JOURNAL OF AGRICULTURAL RESEARCH

VOL. 60 WASHINGTON, D. C., MARCH 15, 1940 No. 6

RELATION OF FOOD TRANSLOCATION TO MOVEMENT OF VIRUS OF TOBACCO MOSAIC [1]

By C. W. BENNETT

Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Previous studies (*2*) [2] have shown that when raspberry plants are infected by the virus of leaf curl or when sugar beet plants are infected by the virus of curly top, virus movement takes place in the phloem of the invaded plants. There is also strong evidence that these two viruses are closely limited to the phloem of their respective host plants and that their movement through the plants is correlated with the translocation of organic food materials. The weight of evidence indicates further that the phloem network of the plant is the avenue through which rapid invasion is accomplished by all viruses that are able to produce systemic infection. Whether the correlation between virus movement and food translocation shown with the two viruses already mentioned exists with other viruses, especially with viruses such as that of tobacco mosaic, which is not limited to the phloem, has not been determined definitely. Conflicting conclusions have been reached from the evidence that has been obtained thus far.

Holmes (*10*) presented evidence that seems clearly to indicate a relationship between the translocation of carbohydrates and the movement of the virus of ordinary tobacco mosaic in tobacco. Samuel (*17*) found an apparent correlation between food translocation and movement of the virus of tobacco mosaic in tomato. Caldwell (*5*, *6*), however, working with the viruses of aucuba and tobacco mosaic in tomato and tobacco, found no correlation between virus movement and food transport. He reached the conclusion that the viruses moved independently of food materials and that under certain conditions virus movement was apparently in directions opposite to that of the metabolites. Grainger (*9*) reached conclusions similar to those of Caldwell and suggested that in his experiments the virus of tobacco mosaic moved equally in all directions in which channels rendered it free to move. He considered that the virus spread through the plant from the point of introduction at a logarithmic rate.

Conflicting conclusions regarding the movement of different viruses led Raber in his review of a book by Curtis (*8*) to suggest that there is no uniformity in the way in which viruses move through the plant, each virus perhaps exhibiting a specific behavior in this respect.

Invasion of plants by viruses is of general interest in the field of plant pathology, and because of the potential significance of plant viruses as indicators of food translocation, virus movement is of

[1] Received for publication June 20, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 389.

particular interest in relation to the general subject of translocation of organic materials in plants. This paper reports the results of experiments on the movement of the virus of tobacco mosaic (tobacco virus 1, Johnson)[3] in *Nicotiana tabacum* L. and *N. glauca* Graham under conditions similar to those under which the movement of the virus of curly top of sugar beet was studied. (*4*).

ACROPETAL AND BASIPETAL MOVEMENT IN STEMS

The early work of Allard (*1*) showed that the passage of tobacco mosaic virus from the point of inoculation near the leaf apex of young tobacco plants to the stem required at least 3 days. Most of the published information on the movement of the virus of tobacco mosaic has been derived from the study of movement in tobacco and tomato plants growing in a normal upright position under more or less normal conditions for photosynthetic activity and food transport. In experiments with tobacco, Holmes (*10*) showed that when the virus was introduced into a leaf its movement was very slow until it entered a vein, after which movement was more rapid and was chiefly in the direction of the stem. After the virus passed out of the inoculated leaf the entire stem was invaded in a relatively short time.

Caldwell (*5*) found that when the virus of tobacco mosaic passed out of an inoculated leaf of a tobacco plant it moved first in the direction of the top. However, Samuel (*17*) found that when the virus moved into the stem of a tobacco or tomato plant it usually moved first in the direction of the root.

Results of numerous tests conducted during the course of the work reported in this paper show that when the virus of tobacco mosaic was introduced into a leaf at the top of a Turkish tobacco plant it moved into the root system within a few days. When the virus was introduced into a leaf near the middle of a stem of a relatively large plant, it moved into the top and the root portions within a period which usually did not exceed 4 to 8 days.

In view of the evidence available, it seems certain that under normal conditions both acropetal and basipetal movements of virus occur in the stems of tobacco and tomato plants within a relatively short time after virus introduction. It should be emphasized, however, that it has not been proved that movements in the two directions occur simultaneously.

It seems probable that when virus enters a stem the direction of its movement may depend upon factors operating in the stem at the time of entry and hence movement may be in either direction, depending on internal conditions in the plant at the time of entry. If virus movement is correlated with food transport, the directional movements that have been observed in the stems of tobacco may readily be explained by assuming that in the normal plant there is a diurnal directional reversal of flow of organic materials in these plants. The virus then would move either acropetally or basipetally, depending on the direction of food flow at the time the virus entered the stem, but it might be carried into the two extremes of the plant in the course of 24 hours.

[3] The virus used in these experiments was obtained from Dr. William N. Takahashi, University of California, who had previously obtained it from Dr. James Johnson, University of Wisconsin.

In plants in which frequent reversals of food flow and virus movement occur, a determination of a possible correlation between movement of the two types of materials would be difficult. However, if a plant could be manipulated in such a way as to insure continuous unidirectional flow of food materials in one of its parts a study of the movement of virus in such a plant would be of interest. With this object in view, the following experiments were made.

EXPERIMENTS WITH NICOTIANA TABACUM

Potted plants of *Nicotiana tabacum*, Turkish variety, about 24 inches tall, were bent so that their stems were in a horizontal position. This resulted in the production of a number of suckers at the base of each stem. One sucker near the base of each plant was retained and the others were removed when small. By this method it was hoped to produce a plant with two growing points, in which the basal shoot, being in an upright position and more advantageously located for growth, would tend to drain the root system of food reserves and prevent outward movement of food materials in the horizontal main stem. Growth of the basal suckers was more rapid in most plants than that of the horizontal stems, suggesting that the basal shoots were deriving a part of their carbohydrate supply from the rest of the plant, at least in the earlier stages of their development.

When the basal suckers were about 2 inches tall the plants were divided into two lots. The plants of the first lot were inoculated on the small leaves at the top of the main stems and the plants of the second lot were inoculated on the basal suckers. The plants were allowed to develop without further treatment, and the time of appearance of symptoms on the two growing points of each plant was noted.

The movement of the virus in a basipetal direction in the main stem was relatively fast, as shown in the results presented in table 1. The entire length of the stem (more than 24 inches) was traversed, and symptoms were produced on the basal sucker in an average period of 6.8 days. This period was only 2 days longer than that required for the production of symptoms on the basal shoots inoculated directly. It may be assumed, therefore, that in this experiment the virus moved basipetally at a rate of approximately 24 inches in 48 hours.

TABLE 1.—*Acropetal and basipetal movement of virus in Nicotiana tabacum and N. glauca*

Species of *Nicotiana*	Plants tested	Place of inoculation	Direction of movement	Average period for appearance of symptoms on—	
				Basal sucker or graft	Growing points at top of plants
	Number			*Days*	*Days*
N. tabacum	20	Top	Toward root	6.8	6.3
	20	Base	Toward top	4.8	35.5
N. glauca	10	Top	Toward root	8.4	5.2
	10	Base	Toward top	5.4	[1] 197.5

[1] 7 of the 10 plants included in this average showed no symptoms on the top graft when the experiment was discontinued 224 to 252 days after the plants were inoculated.

Movement from the inoculated basal suckers acropetally in the main stem was relatively very slow. An average period of 35.5 days was required for the appearance of symptoms in any of the parts of these shoots. During this period the plants had blossomed and produced seed pods that were well advanced toward maturity at the time symptoms were evident.

EXPERIMENTS WITH NICOTIANA GLAUCA

Plants of *Nicotiana glauca* have generally been considered to be able to harbor the virus of tobacco mosaic without manifesting distinct mottling. None of the plants used in these experiments has shown mottling that appeared to be due to the presence of the virus of tobacco mosaic. Since symptoms cannot be relied upon to indicate infection, all experiments on the movement of the virus in this species were conducted with plants in which grafts of Turkish tobacco were placed at the points of inoculation and at the points where it was desired to detect the presence of the virus through expression of symptoms.

Large plants with a single stem were selected for the tests. The stems were cut to a height of 3 feet. Three-inch pieces of stem from healthy Turkish tobacco plants were grafted into the top and base of each plant. The stems were then bent sufficiently to prevent contact between the shoots subsequently produced from the two grafts. When the new shoots from the grafts were 3 to 6 inches long the plants were divided into 2 lots of 10 plants each. Virus was introduced into the basal grafts of 1 lot and into the top grafts of the other. A record of the time of appearance of symptoms on the grafts of each plant was kept.

Table 1 also shows that the basipetal movement from inoculated grafts through the stem of *Nicotiana glauca* was comparable to that in previous experiments with Turkish tobacco. In the plants in which this movement occurred, symptoms appeared on the basal graft in an average time of 8.4 days, 3.2 days later than they appeared on the inoculated grafts of the same plants, and 3 days later than symptoms appeared on directly inoculated basal grafts. If it is considered that this interval represents the time for the virus to move through the *N. glauca* stem from one graft to the other, the rate of movement was 36 inches in about 72 to 77 hours, or about one-half inch per hour.

As in similar experiments with Turkish tobacco (*4*), the acropetal movement was much delayed. The minimum time for the appearance of symptoms on a top graft, in the plants inoculated on the basal graft, was 32 days. In 7 of the 10 plants tested, no symptoms were evident on the top graft in periods ranging from 224 to 252 days.

When the experiment was discontinued, the stems of the seven plants that had produced no symptoms of mosaic on the top graft were severed one-half inch above the point of contact with the basal graft. To determine how far the virus had moved acropetally in the time interval allowed for movement, the stems were cut into 6-inch segments and juice was pressed from each segment and used to inoculate healthy Turkish tobacco plants. A record of the results of the tests from each of these segments is shown in table 2. It is evident from these results that the virus moved toward the top in each of the plants, but the shortness of the distance through which the virus

was able to travel is very striking, especially in those plants in which it was unable to move past the first 6 inches of stem. It is evident that under these conditions movement counter to the direction of predominant food flow was difficult.

TABLE 2.—*Movement of virus from basal grafts of Turkish tobacco upward through stems of plants of Nicotiana glauca*

Plant No.	Period from inoculation of basal graft to testing stem	Presence or absence of virus in indicated 6-inch segment of stem counting from base to top [1]						
		First	Second	Third	Fourth	Fifth	Sixth	Seventh [2]
	Days							
1	224	+	−	−	−	−	−	−
2	225	+	+	−	−	−	−	−
3	225	+	−	−	−	−	−	−
4	225	+	+	+	−	−	−	−
5	250	+	+	+	+	+	−	−
6	251	+	+	−	−	−	−	−
7	252	+	+	+	−	−	−	−

[1] Plus and minus signs indicate virus present or absent, respectively.
[2] 6-inch segment of stem from Turkish tobacco graft at top of plant.

MOVEMENT OUT OF ROOTS

The slow movement of virus acropetally from inoculated basal suckers in Turkish tobacco raised the question whether or not virus would move from the root system in plants under normal conditions of growth. Mulvania (*15*) found that when roots of potted plants were inoculated with the virus of tobacco mosaic no symptoms developed in the tops during the period of observation. Johnson (*11*) obtained similar results with potted plants. Lehman (*12*), in field tests, found that only a very small percentage of plants set in virus-infested soil developed symptoms of mosaic before topping time. Following topping, there was a marked increase in percentage of plants showing symptoms. All of these investigators concluded that infection of roots was rare or did not occur.

However, Price (*16*), in more recent work, concluded that roots of plants of *Nicotiana glutinosa* and Turkish tobacco were susceptible to infection. He was able to obtain relatively high concentrations of virus from roots of some of the inoculated plants 21 days after inoculation although the plants showed no symptoms.

In view of the apparent relationship between virus movement and food translocation it seems possible that these failures to obtain symptoms in root-inoculated plants may have been due to inability of the virus to move from the root system into the top, where symptoms could be expressed.

In order to test this theory, experiments of the following type were made. Small Turkish tobacco plants were placed in 3-inch pots and allowed to grow until they were approximately 6 inches tall. At this stage of development, two or three of the lowest leaves were removed from each plant in order to reduce the chances of accidental infection in later manipulations and the plants were removed from the pots. The root systems had become somewhat pot-bound, and each ball of soil was covered with a fairly compact mass of roots. The roots on the lower half of each ball of soil were inoculated by

rubbing them with a cloth saturated with inoculum. The plants were then placed in 8-inch pots. At the same time other plants were transferred directly, without root inoculation, to 8-inch pots and inoculated on the lowest remaining leaf.

The plants were allowed to develop normally under greenhouse conditions, but care was taken to avoid having any of the leaves come in contact with the soil or with the pots. At 10-day intervals for 40 days, five plants on which root inoculations had been made were cut back, leaving a stem about 2 inches long from which, on most plants, lateral buds soon developed into shoots.

The severed tops were defoliated and the stems were cut into 6-inch segments, which were planted in sand to determine by subsequent growth whether the virus was present at the time the tops were removed. The results of tests of the stem segments of the plants of one experiment are shown in table 3.

TABLE 3.—*Movement of virus out of the roots of Turkish tobacco plants before and after removal of tops*

Plant No.	Treatment after inoculation	Period for appearance of symptoms, counting from—		Presence or absence of virus in indicated 6-inch segment of stem counting from base to top [2]					
		Removal of top [1]	Inoculation	First	Second	Third	Fourth	Fifth	Sixth
		Days	*Days*						
1	Top removed after 10 days.	11	21	+	−				
2		10	20	−	−				
3		21	31	−	−				
4		10	20	−	−				
5		9	19	−	−				
6	Top removed after 20 days.	6	26	+	+	+	+		
7		6	26	−	−	−	−		
8		7	27	−	−	−	−		
9		7	27	−	−	−	−		
10		7	27	−	−	−	−		
11	Top removed after 30 days.	6	36	−	−	−	−	−	−
12		6	36	−	−	−	−	−	−
13		7	37	−	−	−	−	−	−
14		−1	29	+	+	+	+	+	+
15		7	37	−	−	−	−	−	−
16	Top removed after 40 days.	−9	31	+	+	+	+	+	+
17		8	48	+	−	−	−	−	−
18		7	47	−	−	−	−	−	−
19		−5	35	+	+	+	+	+	+
20		7	47	−	−	−	−	−	−
21	Top not removed		67						
22			35						
23			57						
24			63						
25			13						
26	Top not removed; inoculated on lowest leaf.		6						
27			8						
28			6						
29			8						
30			7						

[1] A minus sign preceding a number shows that symptoms appeared on the top of the plant the indicated number of days before the top was removed.
[2] Plus and minus signs indicate virus present or absent, respectively.

A record was kept of the time of appearance of symptoms on all of the inoculated plants. A high percentage of infection was obtained in all lots of plants in which the roots were inoculated. Symptoms, however, were very much delayed, and removal of tops had a decided influence on the time of appearance of symptoms. Table 3 shows in plants 6 to 20, from which the tops were removed 20 to 40 days

after inoculation, that symptoms of mosaic appeared in the new growth of the lateral buds in 6 to 8 days after the tops were removed. In plants 1 to 5, from which the tops were removed 10 days after inoculation, the longer periods required for appearance of symptoms probably were due to very slow growth following removal of the tops.

It may be noted that the plants that were inoculated on the lowest remaining leaf produced symptoms in 6 to 8 days after inoculation, showing that the virus was able to move to the top of the plant from points near the base of the stems in a relatively short time. However, assuming correlation between virus movement and food transport, this movement would be expected if frequent reversals of food movement occurred in the stem. It seems probable that considerable food storage may occur in the base of the stem and even in the larger roots and that at times there would be acropetal movement of these materials. It would be expected, also, that in the distal portion of the root system of a normal plant movement would be unidirectional and toward the root tips. Virus would have difficulty in moving out of small roots under such conditions. Removal of tops, however, might extend the zone in the root system in which movement of foods acropetally could take place. This would account for the rapid movement of virus out of the inoculated roots following removal of the tops of the plants.

EFFECT OF DEFOLIATION ON MOVEMENT

Previous studies (*4*) have shown that the virus of curly top moved upward at a relatively rapid rate in defoliated stems of *Nicotiana glauca* but that upward movement was greatly retarded in foliated stems. Tests were made in an effort to determine the effect of defoliation on the movement of the virus of tobacco mosaic in plants of *N. tabacum*, Turkish variety, and experiments with *N. glauca*, similar to those in which the movement of the virus of curly top was studied, were made with the virus of tobacco mosaic.

EXPERIMENTS WITH NICOTIANA TABACUM

Potted plants of Turkish tobacco 24 to 30 inches tall were bent so that their stems approached a horizontal position. When a selected basal sucker was about 2 inches tall it was inoculated and the main stem was immediately defoliated and pruned to a length of 30 to 36 inches. At intervals of 2 days for 18 days, beginning with the fourth day after inoculation, stems were cut into 3-inch segments and tested for the presence of virus by using extracted juice from each segment to inoculate healthy plants of Turkish tobacco. Five plants were tested at each time interval, and five foliated plants were tested at the end of the experiment as a check on the effect of defoliation on the acropetal movement of the virus.

The extent of the acropetal movement of the virus from the inoculated suckers through the defoliated and foliated stems, as indicated by the tests of stem segments, is shown in table 4. These results show that acropetal movement was relatively fast in defoliated stems and that by the twelfth day the virus had moved the full length of the stem of each plant. Results from the 14-, 16-, and 18-day tests were the same as those from the 12-day tests, and for this reason they are not shown in table 4. The results obtained with the foliated

check plant show that with normal foliage the acropetal movement was much retarded, since in 18 days the virus had not traveled more than 9 inches and in two of the plants it apparently had not entered the first basal 3-inch segment.

TABLE 4.—*Effect of defoliation on acropetal movement of virus in stems of Nicotiana tabacum*

Plant No.	Period from inoculation and defoliation to testing	Presence or absence of virus in indicated 3-inch segment counting from base to top [1]											
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	Twelfth
	Days												
1	4	−	−	−	−	−	−	−	−	−	−	−	
2		−	−	−	−	−	−	−	−	−	−	−	−
3		−	−	−	−	−	−	−	−	−	−	−	
4		+	+	−	−	−	−	−	−	−	−	−	−
5		+	−	−	−	−	−	−	−	−	−	−	−
6	6	−	−	−	−	−	−	−	−	−	−	−	−
7		+	−	−	−	−	−	−	−	−	−	−	−
8		+	+	+	+	+	+	+	+	+	+	+	
9		+	+	+	+	+	+	+	+	+	+	−	−
10		+	+	−	−	−	−	−	−	−	−		
11	8	+	+	+	+	+	+	+	+	+	+	+	+
12		+	+	−	−	−	−	−	−	−	−	−	
13		+	+	+	+	+	+	+	+	+	+		
14		+	+	−	−	−	−	−	−	−	−		
15		+	+	−	−	−	−	−	−	−	−	−	
16	10	+	+	+	+	+	+	+	+	+	+	+	+
17		+	+	+	+	+	+	+	+	+	+	+	+
18		+	+	+	+	+	+	+	+	+	+	+	+
19		+	+	+	+	+	+	+	+	+	+		
20		+	+	+	+	+	+	+	+	+	+	−	−
21	12	+	+	+	+	+	+	+	+	+	+	+	
22		+	+	+	+	+	+	+	+	+	+	+	+
23		+	+	+	+	+	+	+	+	+	+	+	+
24		+	+	+	+	+	+	+	+	+	+	+	+
25		+	+	+	+	+	+	+	+	+	+	+	
26	18 (Check [2])	−	−	−	−	−	−	−	−	−	−	−	−
27		−	−	−	−	−	−	−	−	−	−	−	−
28		+	−	+	−	−	−	−	−	−	−	−	−
29		+	−	−	−	−	−	−	−	−	−	−	−
30		+	−	−	−	−	−	−	−	−	−	−	−

[1] Plus and minus signs indicate virus present or absent, respectively.
[2] Plants not defoliated.

EXPERIMENTS WITH NICOTIANA GLAUCA

Plants with stems 36 inches long and with grafts of Turkish tobacco at the top and base arranged as described in previous experiments with *Nicotiana glauca* were used in further tests of the effects of defoliation on upward movement of virus in stems. When the shoots from the basal grafts of these plants were about 12 inches long, they were inoculated and the plants were left undisturbed for 12 days. At the end of this period, all the inoculated shoots showed distinct symptoms of mosaic and all the grafts at the tops of the plants were normal. After the 12-day period, five of the stems were cut into 6-inch segments and incubated in a moist chamber at room temperature for 24 hours, after which expressed juice from each segment was used to inoculate healthy Turkish tobacco plants. The results from these inoculations indicate the average extent of upward invasion during the first 12 days following the original inoculation (fig. 1, check *a*). All of the other plants, except five (fig. 1, check *b*), that were retained as controls and tested at the end of the experiment, were defoliated

except for the basal inoculated graft. At intervals of 2 days for a period of 18 days, stems were cut into 6-inch segments and tested for virus as already described (fig. 1). On the eighteenth day, the remaining foliated check plants were cut into 6-inch segments and tested for virus. The average distances of virus movement, in inches, in the defoliated stems from the basal inoculated grafts, in comparison with virus movement in the foliated checks *a* and *b*, are shown in figure 1.

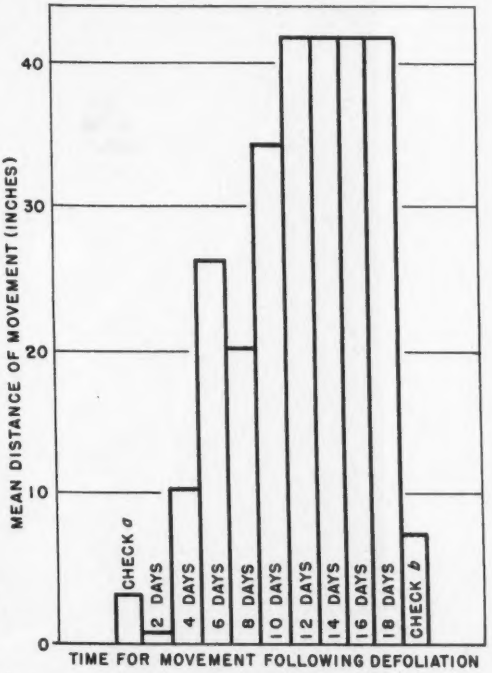

FIGURE 1.—Effect of defoliation on movement of tobacco mosaic virus from infected basal grafts of Turkish tobacco upward through defoliated stems of *Nicotiana glauca* toward top grafts of Turkish tobacco. Results are given as 5-plant averages of data obtained from the tests of 6-inch stem segments taken, at intervals of 2 days, for 18 days after the initial 12-day period allowed for establishment of the virus in the inoculated basal graft. Checks *a* and *b* were not defoliated.

Acropetal movement of virus started in less than 4 days after defoliation of the main stem and progressed rapidly until the entire stem of each of the plants was invaded in 12 days or longer. Figure 2 shows a defoliated plant in which the virus moved to the top and produced distinct symptoms of mosaic within 12 days. The results of the tests of stem segments of the two lots of plants that were not defoliated show that in these plants, growing under more or less normal conditions, relatively little movement acropetally occurred during the course of the experiment. These results demonstrate conclusively that the acropetal movement of virus in the defoliated plants was a direct response to defoliation.

These results reemphasize the fact that under certain conditions acropetal movement of virus into leafy tops of plants, presumably counter to the major movement of elaborated food materials, is difficult and usually very slow as compared to movement in the reverse direction. They show further that, when the carbohydrate-synthesizing areas are removed or reduced so that a food deficit results, movement into the defoliated parts is greatly accelerated.

FIGURE 2.—Effect of defoliation on upward movement of virus. This plant of *Nicotiana glauca* was inoculated on the basal graft of Turkish tobacco; 12 days later all foliage was removed from the main stem. Photographed 12 days after defoliation. Symptoms of mosaic are evident on the rolled leaves of the new growth of the top graft of Turkish tobacco.

When the results shown in figure 1 are compared with the results (*4, fig. 2*) obtained in the study of the upward movement of the curly top virus in the same type of plant under similar conditions, it seems probable that, under the conditions of the tests, movements of the two

viruses were similar and were influenced by the same factors operating approximately equally in each plant.

EFFECT OF DARKNESS ON MOVEMENT

Turkish tobacco plants with main stems in a horizontal position and with basal suckers similar to those already described were used in experiments to test the influence of darkness on acropetal movement of virus. The main stems of the selected plants were 30 to 36 inches long at the time the tests were started.

In the first tests, the main stems of the plants were placed in a dark box but the basal suckers were exposed to the normal light conditions of the greenhouse throughout the tests. The basal suckers were inoculated at the time the main stems were placed in the dark. At intervals of 5 days for 20 days following inoculation, plants were removed from the dark and the main stems were defoliated and cut into 6-inch segments. Juice was pressed from each segment and used to inoculate healthy Turkish tobacco plants to determine the presence or absence of virus.

Movement of the virus in an acropetal direction from the inoculated sucker was slow at first and apparently little movement occurred during the first 5 days (table 5). Movement was more marked during the second and third 5-day periods, and after 20 days virus was found in all the segments of each of the stems of the plants of this series.

TABLE 5.—*Movement of virus in an acropetal direction in darkened horizontally placed main stems of Turkish tobacco plants, from basal upright shoots in the light*

Plant No.	Period from inoculation to testing	Presence or absence of virus in indicated 6-inch segment of stems after indicated period in dark, counting from base to top [1]					
		First	Second	Third	Fourth	Fifth	Sixth
	Days						
1	5	+	−	−	−	−	−
2		+	−	−	−	−	−
3		−	−	−	−	−	−
4		−	−	−	−	−	−
5		−	−	−	−	−	−
6	10	+	−	−	−	−	−
7		+	+	+	+	+	+
8		+	+	+	−	−	−
9		+	+	−	−	−	−
10		+	−	−	−	−	−
11	15	+	+	+	+	+	+
12		+	+	+	+	−	−
13		+	+	+	+	+	+
14		+	+	+	+	+	+
15		+	+	+	+	+	+
16	20	+	+	+	+	+	+
17		+	+	+	+	+	+
18		+	+	+	+	+	+
19		+	+	+	+	+	+
20		+	+	+	+	+	+

[1] Plus and minus signs indicate presence or absence of virus, respectively.

Although comparison of these results with those obtained with similar plants growing under more nearly normal conditions of illumination (table 1) indicates that darkness had a marked effect in inducing acropetal movement, the response in virus movement seems less striking than that previously obtained (*4*) with the virus of curly

top in sugar beet. This difference may be associated in part with yellowing and dying of darkened tobacco leaves, which may have resulted in an outward movement of materials from these leaves during the first few days they were in the dark, and this in turn may have tended to delay the movement of materials, including virus, from the basal parts of the plants.

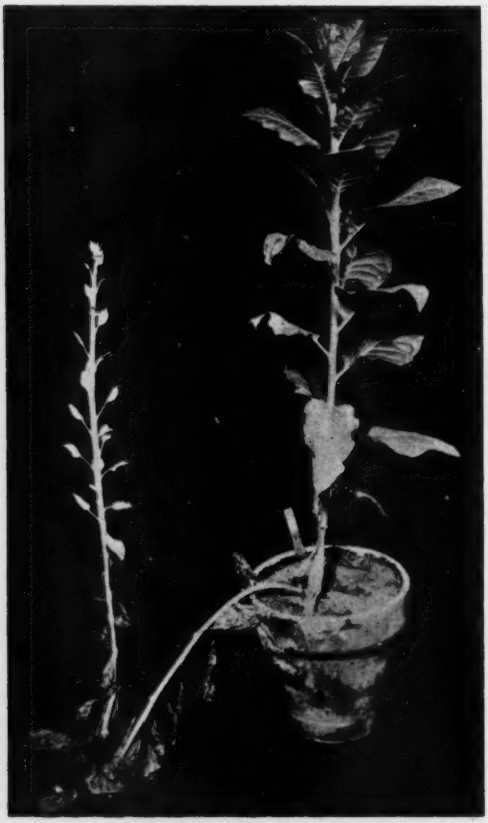

FIGURE 3.—Plant of *Nicotiana tabacum* inoculated on a small leaf at the top of the main shoot immediately after it was placed in the dark. The basal upright shoot was exposed to the light. The plant, photographed 40 days after inoculation, shows the new growth produced in the dark, the dead leaves on the older part of the plant, and the growth produced by the basal sucker during the period of the experiment. The virus did not pass out of the main shoot of this plant in a period of 40 days in the dark.

In a second experiment, the main shoots of plants similar to those used in the experiment just described were placed in a dark box and the basal suckers were exposed to normal light conditions. At the time the shoots were placed in the dark, one of the small leaves at the top of each darkened shoot was inoculated. Check plants were used in which all parts were in the light.

The plants in the dark and the check plants were watched for the appearance of symptoms on the tops of the main shoots and on the basal suckers. Symptoms appeared on the inoculated tops of the check plants in an average period of 5.2 days and on the basal suckers in an average period of 7.8 days.

Owing to the etiolated condition of the tops of the shoots in the dark, symptoms were not distinguished on the inoculated parts of these plants. The minimum time for the appearance of symptoms on a basal sucker was 29 days, and on 7 of 10 plants tested no symptoms were evident on the basal suckers after 40 days. At the end of the 40-day period, it was found that all of the darkened leaves that had normal green color when placed in the dark box had died but that from 15 to 24 inches of new

growth had been produced during the time the shoots were in the dark. Figure 3 shows one of these plants at the time it was removed from the dark.

Upon removal from the dark box after a period of 40 days, the main stems were defoliated and cut into 5-inch segments. Juice was pressed from these segments and used to inoculate healthy Turkish tobacco plants to determine the extent of virus spread from the points of inoculation during the period of test.

The distribution of virus in 7 of the 10 plants used in the experiment is shown in table 6. The results show that in these plants the virus was present in all of the etiolated segments but had moved to the basal sucker in only one plant. Symptoms appeared on the basal sucker of this plant a few days after the main stem was removed. The parts of the remaining 6 plants from which the main stems had been removed were kept for several months. All remained free of symptoms. Thus, in 6 of the 10 plants the virus was unable to pass basipetally out of the darkened shoots in a period of 40 days.

TABLE 6.—*Distribution of virus in inoculated main stems of plants of Turkish tobacco after they had been in the dark for 40 days*

[Each plant had an upright basal shoot that was kept under normal light conditions]

Plant No.	Presence or absence of virus in indicated 5-inch segment of stem, counting from base to top [1]								
	First	Second	Third	Fourth	Fifth	Sixth [2]	Seventh [3]	Eighth [3]	Ninth [3]
1	+	+	+	+	+	+	+	+	+
2	−	−	−	+	+	+	+	+	+
3	−	+	−	+	+	+	+	+	+
4	−	−	+	+	+	+	+	+	+
5	−	−	+	+	+	+	+	+	+
6	−	−	−	+	+	+	+	+	+
7	−	−	−	+	+	+	+	+	+

[1] Plus and minus signs indicate virus present or absent, respectively.
[2] Inoculated segment.
[3] Etiolated segments produced in the dark; the ninth segment ranged from 4 to 9 inches long, depending on the plant.

EFFECT OF SEED PRODUCTION ON MOVEMENT

Vickery et al. (*18*) have shown that at the time the tobacco plant fruits there is an appreciable movement of carbohydrates and other materials from the mature leaves and from the root system into the flower parts. It seems likely that this movement may be extensive enough to cause a more or less uninterrupted movement of materials toward the fruiting parts during the time seeds are being produced. Periodic directional reversal of flow of materials, therefore, may occur only sparingly or not at all at such times in these plants. If this is true and if virus movement is correlated with food flow, it follows naturally that acropetal movement of virus in fruiting plants should be more rapid than in nonfruiting plants and that basipetal movement should be less rapid.

Turkish tobacco plants with horizontally placed stems and with basal suckers as already described were used to test this theory. Four lots of plants were prepared. In the first, the basal sucker was inoculated before the plants had produced blossom buds. To keep the plants in a vegetative condition so far as possible, all fruiting structures were removed before any blossoms opened. Figure 4

shows one of the plants of this lot. In the second, third, and fourth lots the basal suckers were inoculated, respectively, when the first blossom opened, when the first seed pod was full size, and when the most mature seed pod was beginning to turn brown. In all four lots of plants, however, the bending of the main stem was timed so that the basal suckers were less than 6 inches long when they were inoculated.

FIGURE 4.—Type of plant of *Nicotiana tabacum* used to study the acropetal movement of virus in a main stem of tobacco. The basal sucker was inoculated when it was about 2 inches tall and blossom buds were removed from the main stem before any blossoms opened. Photographed 30 days after the basal sucker was inoculated.

The plants of the second, third, and fourth lots were allowed to fruit normally. At intervals of 5 days, following the inoculation of the basal suckers, five plants of each lot were cut into 6-inch segments and each segment was planted in sand in a separate pot. Subsequent growth indicated presence or absence of virus in the segment and showed the extent of invasion of the main stems of the plants in the allotted time intervals.

In these plants the acropetal movement of the virus in the main stem from the inoculated sucker was influenced to a marked degree by seed production. The extent of this influence is illustrated in figure 5. In the plants that were not allowed to blossom the virus failed to reach the top of the main stems in a period of 40 days except in one of the plants in the 30-day lot. In the three lots of plants that were allowed to fruit the virus moved into the tops of all of the plants in periods of 5 to 15 days after appreciable acropetal movement had started.

These results show that conditions are unfavorable for acropetal movement of virus in tobacco plants of the kind tested prior to the time of fruiting, but that there is a period at the time of seed production during which conditions are peculiarly favorable for movement of virus in the direction of the fruiting parts. This period falls within the time during which the plants are transporting appreciable quan-

tities of organic materials to the fruiting parts for the production of seeds.

Since fruiting was correlated with a marked increase in rate of movement of virus from the base of the plant toward the fruiting area, it would seem to follow that movement in the reverse direction, or basipetally, would be less rapid in fruiting plants than in plants in a vegetative condition if virus movement is influenced by the factors responsible for food translocation.

In an experiment planned to test this hypothesis, plants of Turkish tobacco were bent to a horizontal position as in other experiments.

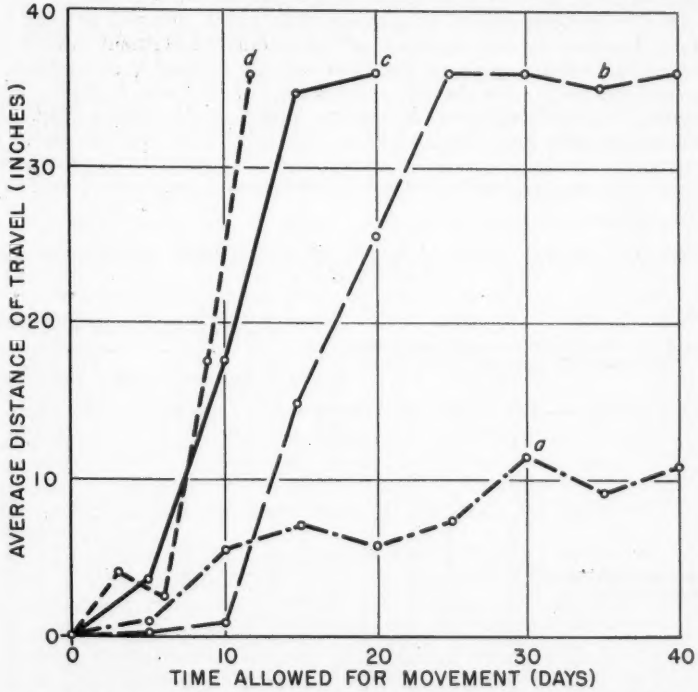

FIGURE 5.—Acropetal movement of the virus of tobacco mosaic in the main stems of plants of *Nicotiana tabacum* into which the virus was introduced through basal suckers when the main stems were in the following stages of development: *a*, Vegetative, not allowed to blossom; *b*, first blossom open; *c*, most mature seed pod full size; *d*, most mature seed pod brown.

When the seed pods of the main stem began to turn brown, leaves of the inflorescence were inoculated. At 5-day intervals for 20 days, stems were severed one-half inch above the point of attachment of the basal sucker, defoliated, cut into 6-inch segments, and tested for the presence of virus by using expressed juice from each segment to inoculate healthy Turkish tobacco plants.

An equal number of check plants were used. These, however, were younger, and when leaves at the distal end of the main stems were inoculated no blossom buds were evident. Stems were severed daily

for 5 days after inoculation, and 6-inch segments were tested for the presence of virus by the method used for the lots of older plants.

The difference in rates of basipetal movement of virus in these two types of plants is striking as shown in table 7, where the results of tests of the segments of each plant are recorded. Movement of the virus in the direction of the root system was very rapid in the vegetative plants, as was expected. In two of these plants the virus moved a distance of 36 inches in 3 days, and in all of the plants tested it moved a distance of 36 inches or more in 4 days. In these plants the virus had either moved the full distance to the roots in the time interval selected or it had not moved out of the inoculated segment.

In the fruiting plants, movement was much delayed and in one plant the virus did not move out of the inoculated segment in 20 days. In five of the fruiting plants the virus was intercepted in its movement toward the root even though the interval of test was 5 days. This suggests that throughout the entire length of the stems basipetal movement may have been slow as compared with that in stems of vegetative plants, where the virus was not intercepted in its movement toward the roots in any instance, although the interval of test was only 1 day.

TABLE 7.—*Influence of stage of maturity of Turkish tobacco plants on basipetal movement of virus*

Stage of development of inoculated plant	Period from inoculation to test for virus	Plant No.	Basal sucker [1]	Presence or absence of virus in indicated 6-inch segment counting from base to top [1]					
				First	Second	Third	Fourth	Fifth	Sixth [2]
	Days	1	−	−	−	−	+	−	+
		2	−	−	−	−	−	−	+
	5	3	−	−	−	−	−	−	+
		4	−	−	−	−	−	−	+
		5	−	−	−	−	−	−	+
		6	−	−	−	−	−	−	+
		7	−	−	−	−	−	−	+
	10	8	+	+	+	+	+	+	+
		9	−	−	−	−	−	−	+
Oldest seed pods beginning to turn brown.		10	−	−	−	−	−	−	+
		11	−	−	−	−	−	+	+
		12	−	−	−	−	−	+	+
	15	13	+	+	+	+	+	+	+
		14	−	−	−	−	+	+	+
		15	−	−	−	−	+	+	+
		16	+	+	+	+	+	+	+
		17	−	−	−	−	−	−	+
	20	18	+	+	+	+	+	+	+
		19	+	+	+	+	+	+	+
		20	+	+	+	+	+	+	+
		21	−	−	−	−	−	−	+
		22	−	−	−	−	−	−	+
	2	23	−	−	−	−	−	−	+
		24	−	−	−	−	−	−	+
		25	−	−	−	−	−	−	+
		26	−	−	−	−	−	−	+
		27	−	−	−	−	−	−	+
Plants wholly vegetative, blossom buds not evident.	3	28	+	+	+	+	+	+	+
		29	−	−	−	−	−	−	+
		30	+	+	+	+	+	+	+
		31	+	+	+	+	+	+	+
	4	32	+	+	+	+	+	+	+
		33	+	+	+	+	+	+	+
		34	+	+	+	+	+	+	+
		35	+	+	+	+	+	+	+

[1] Plus and minus signs indicate virus present or absent, respectively.
[2] Inoculated segment.

RESISTANCE OF DIFFERENT TISSUES OF STEMS TO PASSAGE OF VIRUS

Previous experiments (*3*) have shown that the virus of curly top of sugar beet did not move through portions of stems of *Nicotiana tabacum* and *N. glauca* in which the phloem continuity was broken by ringing. However, the virus was able to move in both the external and internal phloem and to pass from one type to the other through the union of the two in the leaf traces.

The virus of curly top shows evidence of being limited to the phloem, and for this reason probably would not be expected to pass through such structures as medullary rays, wood parenchyma, or other types of parenchymatous tissues. The virus of tobacco mosaic, however, occurs in much of the tissue outside of the phloem and probably multiplies and reaches high concentrations in parenchyma of the common varieties of commercial tobaccos. With these concepts in mind, tests were made to determine the effect of various types of rings in stems of *Nicotiana tabacum* and *N. glauca* on the passage of virus.

EXPERIMENTS WITH NICOTIANA TABACUM

Potted plants of *Nicotiana tabacum* were allowed to grow until they reached the blossom stage, when they were pruned back to a height of about 18 inches. Internal and external rings were then placed in the stem below the second bud counting from the top of the stem. The external ring was made by removing a ring of bark and cambium approximately 2 mm. wide. The internal ring was made by removing a part of the stem by means of a small cork borer and scraping out a ring of pith and internal phloem.

After the rings were made the plants were allowed to stand for 3 or 4 days, when the rings were again scraped and the holes for the internal rings were filled with paraffin having a melting point of 60° C. Leaves on parts above the rings were then inoculated and the plants were observed for the appearance of symptoms on parts above and below the rings.

The position of the rings and the results of the tests are shown in table 8. As indicated, the rings were placed in positions to determine whether the virus was able to move through (1) internal phloem, (2) external phloem, (3) vertically through the woody cylinder, (4) from external to internal phloem through the woody cylinder, (5) from internal to external phloem through the woody cylinder, (6) from internal to external phloem through the phloem connections in the leaf traces, and (7) from external to internal phloem through connections in the leaf traces.

There was no appreciable delay in passage of virus through rings in which there was uninterrupted phloem tissue. Virus passed radially through the woody cylinder in the absence of phloem connections, but apparently passage was delayed an average period of 8 days or more. It seems evident also that the virus was able to travel vertically in the woody cylinder, but travel was rather slow and movement of a distance of little more than 2 mm. apparently required an average time of more than 20 days as indicated by the delay in appearance of symptoms on shoots across the rings. However, it is possible that a part of this apparent delay was caused by the time required for the

virus to move into the tops of the indicator shoots, which had grown considerably during the period of the experiment.

TABLE 8.—*Effect of different types of rings in stems of Nicotiana tabacum on the movement of virus through the ringed parts*

Position of rings	Plants tested	Average period for appearance of symptoms on shoots	
		Above rings	Below rings
	Number	*Days*	*Days*
External ring only	5	4.8	6.1
Internal ring only	5	4.4	5.8
External and internal rings at same level	12	6.2	29.5
External ring 1 inch above internal, both in internode	6	5.0	14.0
Internal ring 1 inch above external, both in internode	5	6.0	13.8
External ring 1 inch above internal, node between	5	5.4	6.6
Internal ring 1 inch above external, node between	5	5.4	6.0

This delay in virus passage may account for the results obtained by Matsumoto and Somazawa (*13*) in which they found that, although the virus of tobacco mosaic occurred in the woody cylinder of tobacco stems, it apparently did not pass portions of the stem from which both internal and external phloem had been removed. If tests for presence of virus were made shortly after symptoms appeared on the inoculated shoots, as may be inferred, it is probable that not enough time was allowed to permit passage of virus through the ringed parts of the stems.

EXPERIMENTS WITH NICOTIANA GLAUCA

An experiment similar to that just described was made with *Nicotiana glauca*, instead of Turkish tobacco, to test the movement of virus through different stem tissues. Plants having single stems were pruned to a height of about 24 inches, and healthy scions of Turkish tobacco were grafted into the base and top of each stem. When the grafts were well established, rings of the same type as those used in Turkish tobacco in the experiment already described were placed in the stems of *N. glauca* a short distance below the top graft. After the rings were made, the top graft of each plant was inoculated and the plants were watched for the appearance of symptoms of mosaic on the grafts of Turkish tobacco above and below the rings.

As in experiments with *Nicotiana tabacum*, rings that did not destroy phloem continuity had no measurable effect on the movement of the virus through the stem. However, the results obtained from the plants in which phloem continuity was broken by the rings were decidedly different from those obtained with *N. tabacum*. In some of the plants (table 9) in which there were no phloem strands bridging the rings, symptoms appeared on the lower graft after considerable delay. Perhaps in these plants the virus passed the rings in the absence of phloem. However, it is recognized that in experiments of this type there is always danger of accidental infection, since healthy shoots of the very susceptible Turkish tobacco were growing in close proximity to infected parts.

TABLE 9.—*Effect of different types of rings in the stem of Nicotiana glauca on passage of the virus of tobacco mosaic*

Position of rings	Plants infected	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period from inoculation to appearance of symptoms below ring	Plants affected	Average period from inoculation to discard of plants
	Number	*Number*	*Days*	*Number*	*Days*
No ring	5	5	7.8		
External ring only	5	5	8.6		
Internal ring only	5	5	6.4		
External and internal rings at same level	5	1	19.0	4	376
External ring 1 inch above internal, both in internode	5	3	104.5	2	390
Internal ring 1 inch above external, both in internode	4	3	46.3	1	327
External ring 1 inch above internal, node between	5	5	8.2		
Internal ring 1 inch above external, node between	5	5	7.8		

Regardless of what may have happened in the stems of the plants in which symptoms of mosaic appeared on both sides of the rings, it seems clear that at least some of the plants were able to restrict the virus to a certain portion below which phloem continuity was severed. In some of these plants the virus was held in the parts above rings for periods of several months. One of these plants is shown in figure 6. A more detailed view of another plant of this series is shown in figure 7.

At the time the plants in which the virus failed to pass the rings were discarded, transverse sections of the stems in the ringed portions were examined to determine whether living cells connected the parts above and below the rings. Where the rings were at the same level, the exposed inner and outer tissue enclosed a part that seemed similar to normal wood. Living wood parenchyma cells were present, some with chloroplasts. Where the rings were at different levels, the pith, wood, and bark appeared normal between the rings, and numerous living strands bridged the space between the inner and outer phloem. From these observations it was concluded that there were bridges of living tissue connecting the phloem of the upper infected part with the lower noninfected part in all of the plants in which the virus failed to pass the rings.

These results seem to justify the conclusion that in at least some of the plants of *Nicotiana glauca* the living parenchyma of the stem is able to act as a barrier to the passage of the virus of tobacco mosaic. In such stems both the medullary rays and the wood parenchyma are impermeable to the virus.

MOVEMENT OF THE VIRUS OF CUCUMBER MOSAIC THROUGH RINGS IN THE STEMS OF NICOTIANA GLAUCA

After it was found that the virus of tobacco mosaic was unable to move longitudinally or radially through the woody cylinder of certain plants of *Nicotiana glauca* in the rather long time interval allotted for movement, experiments with a second virus were started. The virus

FIGURE 6.—Restriction of virus movement in stems of *Nicotiana glauca* by rings that destroyed phloem continuity. Grafts of Turkish tobacco were placed at the top and base of the stem. External and internal rings (positions shown by arrows) were placed at different levels in an internode, and the top graft was inoculated September 18, 1936. The plant was photographed April 14, 1937. The mosaic symptoms in the top graft are in striking contrast to the normal growth from the basal graft, showing that the virus was unable to traverse the woody cylinder of the stem, vertically or radially.

FIGURE 7.—More detailed view of plant of same series as that shown in figure 6. Photographed 6 months after the top graft of Turkish tobacco was inoculated with the virus of tobacco mosaic. The virus did not pass the rings (positions shown by arrows) in the stem of *Nicotiana glauca* in a period of 6 months, as indicated by the healthy foliage at the top of a shoot from the basal graft of Turkish tobacco shown in the lower part of the figure.

selected for these tests occurs in many of the plants of *N. glauca* growing wild in the vicinity of Riverside, Calif. This virus is able to attack a number of plant species and causes very distinct mottling on *N. glauca* and on Turkish tobacco, although on the latter mottling is much less pronounced than that caused by the virus of tobacco mosaic. Its properties and host range, so far as determined, indicate that it is a strain of the virus of cucumber mosaic common in the United States.

Test plants were prepared as already described in the experiments with the virus of tobacco mosaic. Stems of plants of *Nicotiana glauca* were cut back to a height of about 24 inches and grafts of Turkish tobacco were placed at the top and base. After the grafts were well established, rings were placed in the stems of the *N. glauca* plants about 1 to 2 inches below the top graft. These rings are listed in table 10 and were designed to determine (1) movement through the normal uninjured stem, (2) longitudinal movement through the woody cylinder, (3) radial movement through the woody cylinder, and (4) movement through the phloem of the leaf traces. After the rings were made, the top graft of each plant was inoculated.

TABLE 10.—*Effect of different types of rings in the stem of Nicotiana glauca on movement of the virus of cucumber mosaic*

Position of rings	Plants tested	Average time for appearance of symptoms on grafts	
		Above rings	Below rings
	Number	*Days*	*Days*
No ring	5	20.2	24.6
External and internal ring at same level	13	19.3	43.3
External and internal rings at different levels, both in internode	28	15.5	39.3
External and internal rings at different levels, node between the two rings	5	19.8	20.4

The symptoms produced in these inoculated plants varied considerably, especially on Turkish tobacco. However, they were distinct enough to be detected readily under favorable conditions. The incubation period of the virus was also subject to much variation in different plants and was much longer than that of the virus of tobacco mosaic in Turkish tobacco plants used in earlier experiments.

Average periods for the appearance of symptoms on the inoculated parts and on the parts below the rings are shown in table 10. In these tests the virus moved through the ringed portions of the stems regardless of the presence or absence of phloem connections. Rings that did not sever phloem continuity had no detectable effect on the rate of virus passage, whereas rings that severed phloem continuity caused marked delays in the time of appearance of symptoms on the lower grafts. This indicates a period of slow movement through the woody cylinder.

Another series of plants of *Nicotiana glauca* with top and basal grafts of Turkish tobacco was prepared for study of the effect of rings on passage of the viruses of tobacco mosaic and cucumber mosaic following simultaneous introduction of the two viruses into parts above double rings. After the grafts of these plants were well established, external and internal rings were placed at different levels in an inter-

FIGURE 8.—Plant of *Nicotiana glauca* with top and basal grafts of Turkish tobacco and rings (positions shown by arrows) breaking the continuity of the phloem in the stem of *N. glauca*. The viruses of tobacco mosaic and cucumber mosaic were introduced simultaneously into the top graft. The virus of cucumber mosaic moved through the ringed area and produced symptoms in a period of 37 days. The virus of tobacco mosaic failed to move through the ringed area in 100 days.

node in the stem of *N. glauca* about 2 inches below the lower end of the top graft. Each top graft was then inoculated with both viruses and all grafts were watched for the appearance of symptoms characteristic of those produced by each of the two viruses.

In all of the 20 plants tested, the virus of cucumber mosaic moved through the rings, and symptoms were evident on the foliage below the rings in an average period of 36 days. In 4 plants, symptoms of tobacco mosaic appeared on the lower grafts in an average period of 68 days.

No symptoms of tobacco mosaic were evident on the foliage of the lower grafts of the remaining plants in a period of 100 days, when the experiment was discontinued. Figure 8 shows one of these plants photographed 100 days after it was inoculated, and figure 9 shows

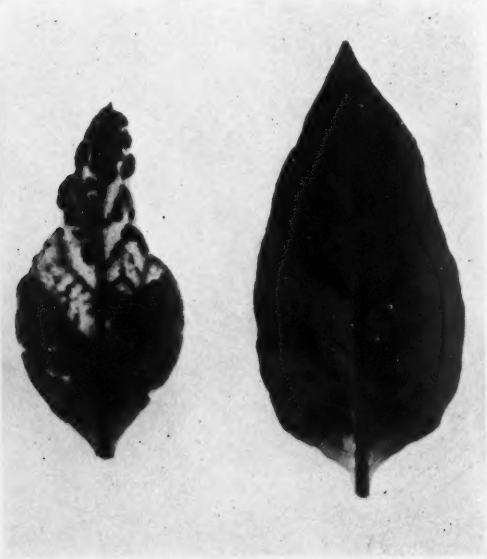

FIGURE 9.—Leaves of Turkish tobacco taken from the top and basal grafts on the plant of *Nicotiana glauca* (fig. 8) in the stem of which the virus of cucumber mosaic passed double rings that destroyed phloem continuity and the virus of tobacco mosaic failed to pass such rings: *A*, From top graft; *B*, from basal graft.

characteristic symptoms produced on leaves taken from the top and basal grafts at the time the plant was photographed.

At the end of the experiment, portions of the top and basal grafts of each plant were removed and dried to destroy the virus of cucumber mosaic. Extracts were prepared from the dried material and used to inoculate healthy tobacco plants. No infection was obtained from extracts from material taken from the basal grafts that did not show symptoms of tobacco mosaic.

These results show that certain plants of *Nicotiana glauca* separated the virus of cucumber mosaic from the virus of tobacco mosaic by virtue of the fact that the former moved through the woody cylinder

of *N. glauca* in the absence of phloem, under conditions which caused the restriction of the virus of tobacco mosaic to the inoculated parts above the rings.

DISCUSSION

In connection with the general subject of factors involved in the movement of viruses in plants, a comparison of the movement of the virus of tobacco mosaic in *Nicotiana tabacum*, Turkish variety, and in *N. glauca* with the movement of the virus of curly top (*4*) in these two species of *Nicotiana* is of interest.

The virus of curly top probably is closely limited to the phloem, but in the common varieties of tobacco the virus of mosaic evidently occurs in both phloem and parenchyma. Certain differences in the ability of two such viruses to invade plants would be expected to result from these different tissue relationships. The differences that appear most evident in the results obtained involve ability of the virus of mosaic to move through rings in the stems of plants of Turkish tobacco in the absence of continuous phloem paths, and in an apparently greater tendency on the part of the virus of mosaic to move in directions opposite food transport under certain conditions.

Before considering these differences further it should be emphasized that there is evidence of two distinct types of virus movement in plants. One type occurs in the parenchyma and the other in the phloem. The factors responsible for movement through the parenchyma probably are diffusion and protoplasmic streaming, perhaps aided by virus multiplication. Movement resulting from the operation of these factors would necessarily be relatively slow and independent of other materials being transported.

The factors responsible for virus movement in the phloem are not well understood, but it is quite evident that under favorable conditions viruses may move through the phloem at very rapid rates. These rates greatly exceed any movements that have been shown to occur in parenchyma.

When the respective tissue relations of the viruses of tobacco mosaic and curly top are considered in relation to the two types of movement that occur in plants, the probable reasons for the observed differences in the movement of the two viruses are more clearly evident.

Since the virus of mosaic is able to move and increase in the parenchyma, it would be expected to pass rings that destroy phloem continuity in the stems of Turkish tobacco. Matsumoto and Somazawa (*13*) showed that the virus occurs in the woody cylinder of tobacco stems. Since there is good evidence that it does not occur ordinarily in nonliving tissue, its presence in the woody cylinder would indicate occurrence in the wood parencyhma and in the medullary rays of the woody portions of the stem. Therefore, the virus should be able to pass rings in stems by traveling radially through medullary rays or vertically through wood parenchyma. But since travel through cells of these tissues would be slow, considerable delay in passage of rings that break phloem continuity would be expected. The results of virus passage through rings in Turkish tobacco show this expected delay.

The same principles may be used to explain the movement of a virus, such as that of tobacco mosaic, in directions opposite food flow. Comparison of the movement of the virus of curly top (*4*) with that of

tobacco mosaic through a horizontal stem of Turkish tobacco acropetally from an inoculated basal sucker shows that the curly top virus had in most instances either reached the top in the time interval allowed or had not moved a distance of one-half inch from the base of an inoculated sucker; whereas in the same type of plant there was a certain amount of evidence of a slow acropetal movement of the mosaic virus throughout the interval allowed for movement. If the virus of curly top is limited to the phloem it would be able to move in the phloem only when conditions were favorable for movement. Evidence indicates that conditions are not usually favorable for movement in phloem in an acropetal direction in the type of plants tested; but if they do become favorable, movement may be very rapid.

However, since the virus of mosaic is able to move in the parenchyma and since this type of movement is independent of movement in the phloem, the virus of mosaic would be able to move acropetally throughout the time allowed for movement at the rate it is able to maintain in parenchymatous cells. Although this rate of movement is not great, it would be detectable in plants held for long periods and might be appreciably greater than that in the phloem under conditions unfavorable for movement in the phloem.

The question naturally arises as to why the tobacco mosaic virus did not in all instances pass certain types of rings in *Nicotiana glauca* in which passage would have involved travel through either the medullary rays or the wood parenchyma. Not enough information is available at this time to answer this question with certainty, but the lack of mottling in infected plants may have some significance in this connection. It is conceivable that the absence of mottling in *N. glauca* was due to a low concentration or absence of virus in the chlorophyll-bearing cells. If this is true, it seems probable that much of the extraphloem tissue of the stem may be unfavorable for multiplication or movement and that the virus may be more or less closely restricted to the phloem, at least in the stem of certain individual plants. It might for this reason be incapable of traveling longitudinally through the wood parenchyma or radially through the medullary rays connecting the regions of internal and external phloem.

This concept is strengthened somewhat by the fact that the virus of cucumber mosaic, which is capable of causing distinct mottling in *Nicotiana glauca* and therefore presumably occurs in relatively high concentration in parenchyma, was able to pass rings in stems of *N. glauca* which destroyed phloem continuity and through which the virus of tobacco mosaic failed to pass.

The evidence obtained with tobacco mosaic virus in the two species of tobacco indicates that the tissue relationships of a virus may be quite different in two distinct host plants.

Similarities in the movement of the virus of tobacco mosaic and the virus of curly top are obvious when movement through the phloem is considered.

According to tests in which plants were inoculated at the top and then segmented after certain intervals, the maximum rate of movement through the phloem determined for the virus of mosaic was 36 inches in 72 hours. Evidence indicates consistently that this rate, of about one-half inch per hour, is maintained in plants under favorable conditions for movement. The virus of curly top apparently moves

at approximately the same rate in the stems of tobacco under similar conditions. Its movement in beet, however, is much more rapid.

It is recognized that there is an element of error in the determination of rates of movement of these viruses in tobacco because the time that movement begins in the phloem cannot be determined accurately. The actual maximum movement, therefore, is somewhat greater than that indicated, but probably less than twice as great. Since the element of error is present in the determination of the movement of each virus, and since its magnitude is not definitely known in either instance, accurate comparisons of the maximum movements in the stem of tobacco are not practicable. However, the evidence clearly indicates that the movement of the two viruses through the phloem of the stem of tobacco is approximately of the same order. It is evident also that both viruses move rapidly in directions of food transport and very slowly in directions opposite food transport.

The way in which the virus of tobacco mosaic moves in defoliated stems, in shoots placed in the dark, and in plants during the period of seed development, indicates clearly that movement of this virus through the stems of plants of Turkish tobacco and of *Nicotiana glauca* can be influenced, and to a considerable extent controlled, by controlling certain factors that determine the direction of movement of elaborated food materials. This seems to furnish very strong evidence in support of the view that virus movement is correlated with the translocation of elaborated food materials in the plants in which tests were made.

In view of this fact it may be well to consider further the evidence that has been interpreted as opposed to such a correlation. This evidence consists principally of the following observations. The virus moves acropetally and basipetally in the stems of normal upright tobacco and tomato plants (*5*, *7*, *9*, *10*). It apparently does not move into darkened mature tobacco leaves more rapidly than it moves into normal leaves in the light, and it moves out of darkened mature leaves almost as readily as it moves out of normal leaves in the light (*5*). It moves out of young leaves before they are mature (*6*).

To interpret this evidence as opposed to the concept of correlated virus movement and food translocation, obviously the following assumptions must be made: (1) Food movement in at least the lower portions of normal upright tobacco and tomato plants is always in the direction of the roots; (2) movement of food materials is into and not out of mature tobacco leaves in the dark; and (3) no food moves out of immature tobacco leaves. It seems very doubtful that these assumptions are justified by the evidence that is available.

Since Samuel (*17*) found that in the stems of tomato plants the virus of tobacco mosaic usually moved first to the roots and later to the tops and Caldwell (*5*) found that in tobacco the virus moved first to the tops, it seems probable that movement in the stem may be in either direction, depending on conditions at the time of entrance.

It is possible that frequent directional reversals of food movement in tobacco and tomato stems may occur under normal conditions of growth and that the observed movements of virus may be correlated with diurnal or other directional reversals of food movement. The slow acropetal movement of the virus of tobacco mosaic in plants of Turkish tobacco and *Nicotiana glauca* manipulated in such a way as

presumably to decrease greatly the acropetal movement of food materials lends support to this idea.

Failure of virus to move into darkened noninoculated mature leaves of tobacco and failure of inoculated darkened leaves to restrict outward movement of virus may have an explanation that does not conflict with the concept of a correlation with food movement. It has been observed repeatedly that when mature leaves of tobacco plants are placed in the dark they soon turn yellow and die. It seems probable that such leaves do not utilize food from parts in the light, for if they did they would continue to live. Owing first to the outward movement of food reserves and later to the outward movement of products resulting from protoplasmic disintegration, the directional movements of materials in the phloem of darkened leaves of tobacco may be similar to those of normal leaves. If this is true, the directional virus movements that have been observed are those that would be expected. Results from experiments in which entire shoots were placed in the dark and in which subsequent growth showed that materials were moved into darkened shoots from parts in the light prove not only that virus can be caused to enter parts by keeping them in the dark but also that virus moves out of such parts with considerable difficulty.

No information is available regarding the stage of development that a tobacco leaf must attain before it becomes a source of supply of carbohydrates to the rest of the plant, but it seems reasonable to suspect that under favorable conditions for photosynthesis temporary surpluses of food materials may be developed in leaves long before they are mature and that at times there may be movement of materials out of relatively young leaves. Such a movement, even if it were a very limited one, would account for the observed movements of virus out of relatively young leaves.

It seems reasonable to conclude that the movement of the virus of tobacco mosaic through the phloem network of the plant is similar to that of the virus of curly top. The transport of these two viruses through the plant is probably the result of the operation of factors responsible for the translocation of elaborated food materials. The observed movements through the plant are of the type that would be expected if the basic principles of the theory of mass flow (*7*, *14*) of liquid materials in the phloem were operating in the translocation of organic food substances.

SUMMARY

In vegetative plants of Turkish tobacco having a main stem in a horizontal position and a basal sucker in a vertical position, basipetal movement of the virus of tobacco mosaic in the main stem was rapid and acropetal movement was slow. In similar plants maturing seeds on the main stem acropetal movement was rapid. In vegetative plants, acropetal movement was accelerated by darkness and defoliation. Basipetal movement was very slow in main stems in the dark.

In plants of *Nicotiana glauca* having top and basal grafts of Turkish tobacco separated by 3 feet of stem, virus moved from the top graft to the basal graft and produced symptoms in 6 to 9 days. In 7 of 10 plants the virus failed to move from the basal to the top graft in periods ranging from 224 to 252 days. Upward movement was relatively rapid when the tops were defoliated.

Roots of Turkish tobacco were susceptible to infection, but usually long periods were required for the virus to move from the roots into the tops and produce symptoms. Removal of the tops hastened the upward movement.

The virus of tobacco mosaic moved through the rings that broke phloem continuity in the stems of Turkish tobacco, but passage was delayed. In certain plants of *Nicotiana glauca*, the virus failed to pass rings that broke phloem continuity in periods some of which were more than 250 days. However, the virus of cucumber mosaic was able to pass such rings in all cases, although passage was delayed by the rings.

Since the virus of tobacco mosaic usually does not produce mottling on *Nicotiana glauca*, it is suggested that the parenchymatous tissue of this species may be unfavorable for movement and multiplication. This may account for the inability of the virus to pass rings that break phloem continuity. The fact that the virus of cucumber mosaic, which causes mottling on *N. glauca* and therefore probably occurs abundantly in parenchyma, passed such rings in all instances lends support to this concept.

The evidence indicates that the movement of the virus of tobacco mosaic is correlated with food transport. The factors involved in movement are probably not fundamentally different from those responsible for the movement of other plant viruses.

LITERATURE CITED

(1) ALLARD, H. A.
1914. THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bul. 40, 33 pp., illus.

(2) BENNETT, C. W.
1927. VIRUS DISEASES OF RASPBERRIES. Mich. Agr. Expt. Sta. Tech. Bul. 80, 38 pp., illus.

(3) ———
1934. PLANT-TISSUE RELATIONS OF THE SUGAR-BEET CURLY-TOP VIRUS. Jour. Agr. Res. 48: 665–701, illus.

(4) ———
1937. CORRELATION BETWEEN MOVEMENT OF THE CURLY TOP VIRUS AND TRANSLOCATION OF FOOD IN TOBACCO AND SUGAR BEET. Jour. Agr. Res. 54: 479–502, illus.

(5) CALDWELL, JOHN.
1934. THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS V. THE MOVEMENT OF THE VIRUS AGENT IN TOBACCO AND TOMATO. Ann. Appl. Biol. 21: 191–205, illus.

(6) ———
1936. THE AGENT OF VIRUS DISEASE IN PLANTS. Nature [London] 138: 1065.

(7) CRAFTS, ALDEN S.
1931. MOVEMENT OF ORGANIC MATERIALS IN PLANTS. Plant Physiol. 6: 1–41, illus.

(8) CURTIS, OTIS FREEMAN.
1935. THE TRANSLOCATION OF SOLUTES IN PLANTS: A CRITICAL CONSIDERATION OF EVIDENCE BEARING UPON SOLUTE MOVEMENT. 273 pp., illus., New York and London. [Reviewed by Oran Raber in Phytopathology 26: 296–298. 1936.]

(9) GRAINGER, J.
1933. THE MOVEMENT OF TOBACCO MOSAIC VIRUS IN ITS HOST. Ann. Appl. Biol. 20: 236–257, illus.

(10) HOLMES, F. O.
1932. MOVEMENT OF MOSAIC VIRUS FROM PRIMARY LESIONS IN NICOTIANA TABACUM L. Boyce Thomp. Inst. Contrib. 4: 297–322, illus.

(11) JOHNSON, JAMES.
1937. FACTORS RELATING TO THE CONTROL OF ORDINARY TOBACCO MOSAIC. Jour. Agr. Res. 54: 239–273, illus.
(12) LEHMAN, S. G.
1934. CONTAMINATED SOIL AND CULTURAL PRACTICES AS RELATED TO OCCURRENCE AND SPREAD OF TOBACCO MOSAIC. N. C. Agr. Expt. Sta. Tech. Bul. 46, 43 pp., illus.
(13) MATSUMOTO, TAKASHI, and SOMAZAWA, KOETSU.
1933. IMMUNOLOGICAL STUDIES OF MOSAIC DISEASE. III. FURTHER STUDIES OF THE DISTRIBUTION OF ANTIGENIC SUBSTANCES OF TOBACCO MOSAIC IN DIFFERENT PARTS OF HOST PLANTS. Soc. Trop. Agr. Jour. 5: 37–43, illus.
(14) MÜNCH, ERNST.
1930. DIE STOFFBEWEGUNGEN IN DER PFLANZE. 234 pp., illus. Jena.
(15) MULVANIA, MAURICE.
1930. ROOT INOCULATION WITH THE VIRUS OF TOBACCO MOSAIC. (Abstract) Jour. Bact. 19: 23–24.
(16) PRICE, W. C.
1938. STUDIES ON THE VIRUS OF TOBACCO NECROSIS. Amer. Jour. Bot. 25: 603–612, illus.
(17) SAMUEL, G.
1934. THE MOVEMENT OF THE TOBACCO MOSAIC VIRUS WITHIN THE HOST. Ann. Appl. Biol. 21: 90–111, illus.
(18) VICKERY, H. B., PUCHER, G. W., LEAVENWORTH, C. S., and WAKEMAN, A. J.
1936. CHEMICAL INVESTIGATIONS OF THE TOBACCO PLANT. V, CHEMICAL CHANGES THAT OCCUR DURING GROWTH. Conn. Agr. Expt. Sta. Bul. 374, pp. [557]–619, illus.

ORGANIC RESERVES IN THE ROOTS OF BINDWEED [1]

By C. G. BARR [2]
Associate in botany, Colorado Agricultural Experiment Station

INTRODUCTION

Organic reserves in the roots of perennial weeds are closely associated with their persistence. Earlier work on bindweed control involved mainly the study of the effect of herbicides and the development of control methods by cultivation or smother crops with little consideration of the physiology of the weed. In 1936 work was undertaken at this station to study the carbohydrate reserves in bindweed roots and their role in growth and regeneration. The experiments reported in this paper relate to the effect of various cultural practices and chemical treatments on the trend of organic reserves in the roots of bindweed (*Convolvulus arvensis* L.).

MATERIALS

The study of the root reserves of bindweed is one phase of a larger program of weed investigations being carried on at the Colorado Agricultural Experiment Station. A 10-acre field of bindweed-infested land is under lease, and various cultural and chemical methods of weed control are in progress. The general plan of this part of the study involved the cultivation of plots at various intervals after emergence of the bindweed following each cultivation. Cultivations during the season of 1936 were (1) at emergence, (2) 3, (3) 6, (4) 9, (5) 12, and (6) 15 days after emergence. In addition to the clean cultivation and chemical tests, various cropping practices are being carried out.[3]

The study of the root reserves consisted essentially in determining the trend of the carbohydrates in the roots of plants under various treatments in comparison with undisturbed plants. Four separate types of collections were made:

1. Root samples collected at biweekly intervals from April 25 to October 30. Two series of plots were sampled—one in which the plants were undisturbed throughout the season, and the other in which the plants were clean-cultivated all season at 9-day intervals after emergence of the bindweed. First emergence of plants commonly occurred about 6 days after cultivating. Therefore cultivating 9 days after emergence is comparable to cultivation once every 2 weeks.

2. Root samples collected at 4-day intervals for a period of 24 days after a single cultivation at various dates during the season.

3. Root samples collected from plots treated on various dates with dry sodium chlorate at the rate of 3 pounds per square rod. Root

[1] Received for publication July 12, 1939. Contribution from the Colorado Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Aid by Bruce J. Thornton, associate in botany, is acknowledged in supplying some of the material for table 6. He also made possible the field work incident to the preparation of this paper. Thanks are also due Dr. L. W. Durrell, head of the Department of Botany, for helpful advice and criticism in preparation of the manuscript.

[3] Under the supervision of Bruce J. Thornton.

samples were taken June 1, July 1, August 1, and September 1 at time of treatment, and also 1 month after treatment. Checks were run on untreated plots.

4. Root samples were collected at the end of the growing season near October 1 from plots as follows:

(1) Uncultivated; (2) cultivated at emergence to depth of 3 inches, April to October; (3) cultivated 3 days after emergence, April to October; (4) cultivated 6 days after emergence, April to October; (5) cultivated 9 days after emergence, April to October; (6) cultivated 12 days after emergence, April to October; (7) cultivated 15 days after emergence, April to October; and (8) cultivated to a depth of 6 inches 9 days after emergence, April to October.

METHOD OF SAMPLING

Root samples were obtained by sifting the soil containing bindweed roots and placing the roots with some soil, in quart mason fruit jars for transport to the laboratory. A post-hole digger which made a hole 6 inches in diameter was used in digging the roots. By making four adjacent holes in the pattern of a square a hole was produced 1 foot square and as deep as required. For the work on bindweed, root samples were taken from the first foot level only. Five cubic feet of soil from five different locations in the plot were taken as a standard composite field sample.

The roots were taken to the laboratory as soon as possible after digging, the soil and sand removed, and the roots washed with cold water. The excess water was removed by blotting with cheesecloth. Duplicate 50-gm. samples were weighed to ± 0.05 gm. on a torsion balance. These were cut into pint mason fruit jars containing 200 ml. of boiling 95-percent alcohol, sealed, and allowed to simmer in a boiling water bath for 45 to 60 minutes. The samples were then stored until they could be conveniently extracted and analyzed.

METHODS OF ANALYSIS

The preserved root material was extracted by decantation with 80-percent boiling alcohol until reducing substances had been removed from the sample as determined by tests. From 12 to 15 extractions were required. The extract from each sample was collected in a volumetric flask and the extracting liquor made to volume after completion of the extraction. Two-hundred-milliliter portions of this liquor were used for the sugar determinations. The alcohol was removed by evaporation and the resulting water solution cleared with neutral lead acetate. Reducing substances were determined by the Munson-Walker (*22*)[4] method for the precipitation of the cuprous oxide. A modification of the Bertrand (*7*) method was used for the estimation of copper. The residue, after the removal of the soluble carbohydrates, was dried to constant weight at 100°C., ground to suitable fineness, and analyzed for starch and acid-hydrolyzable materials separately.

One-gram samples of the residue were gelatinized and digested with undiluted taka-diastase until negative iodine tests for starch were obtained. The digest was then filtered, the filtrate cleared with neutral lead acetate, and the glucose equivalent after hydrolysis was determined on aliquots. The starch fraction included small amounts

[4] Italic numbers in parentheses refer to Literature Cited, p. 412.

of other substances which might be brought into solution by the gelatinizing process and the taka-diastase digestion. Evidence for this is the fact that appreciable values for starch were obtained on samples from fallowed plots when iodine failed to give positive tests for starch. No important reserve material remained in the residue after the alcohol extraction and diastase digestion. This is shown by the uniformly low acid-hydrolyzable values.

The term "readily available carbohydrates," as used in this paper, includes the total sugars and starch only, since acid-hydrolyzable substances probably do not serve as a readily available reserve.

The acid-hydrolyzable fraction is shown in the tables, however, for the convenience of readers who desire to estimate the total carbohydrates. The reducing sugars are necessarily a part of the total sugars as determined after invertase hydrolysis. Results shown in all the tables are expressed on the basis of both fresh and dry weight. The graphs show the data on the basis of fresh weight.

PRESENTATION AND DISCUSSION OF RESULTS

Reserve food in the bindweed roots studied in these experiments occurred chiefly in the form of sucrose and starch. Although the reducing-sugar content was low, approximately 0.50 to 2.00 percent, the total sugars varied from about 2.00 to 7.00 percent. In a recent paper Bakke, Gaessler, and Loomis (*4*) showed that reserves in the bindweed roots of their experiment consisted largely of sucrose and a dextrinlike compound or group of compounds. They showed also that reducing sugars and true starch played less important roles.

TABLE 1.—*Seasonal variations in the carbohydrate content (percent) of cultivated and of undisturbed bindweed roots expressed on fresh-weight and dry-weight bases 1936*

Date	Basis	Undisturbed plants—					Cultivated plants—				
		Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
Apr. 25	Fresh weight	0.53	2.44	1.80	3.12	4.24	------	------	------	------	------
	Dry weight	3.16	14.60	10.81	18.70	25.41	------	------	------	------	------
May 9	Fresh weight	.51	1.91	.93	3.10	2.84	------	------	------	------	------
	Dry weight	3.30	12.36	6.00	19.95	18.36	------	------	------	------	------
May 25	Fresh weight	.66	2.25	1.65	2.50	3.90	0.53	1.50	2.01	2.89	3.51
	Dry weight	4.48	15.13	11.28	16.92	26.41	3.09	8.68	11.65	16.77	20.33
June 8	Fresh weight	1.07	2.54	3.31	2.37	5.85	.40	1.77	1.40	3.00	3.17
	Dry weight	7.13	17.77	22.09	15.85	39.86	2.48	11.00	8.56	18.70	19.56
June 22	Fresh weight	.84	2.81	2.97	2.98	5.78	.26	1.19	.70	3.69	1.89
	Dry weight	4.85	16.34	15.50	17.30	51.84	1.54	6.94	4.06	21.47	11.00
July 6	Fresh weight	.77	2.66	4.20	3.20	6.86	.03	.82	.97	3.20	1.79
	Dry weight	3.95	13.78	21.03	16.57	34.81	.18	5.02	5.97	19.40	10.99
July 20	Fresh weight	1.31	3.94	4.74	3.15	8.68	.01	.70	.78	3.00	1.48
	Dry weight	6.81	18.93	26.50	15.92	45.43	.09	4.52	5.22	20.17	9.74
Aug. 1	Fresh weight	1.21	3.96	6.21	3.50	10.17	.02	.71	.79	3.33	1.50
	Dry weight	5.24	17.05	26.81	15.10	43.86	.03	4.32	4.87	21.60	9.19
Aug. 17	Fresh weight	1.25	2.79	5.58	3.48	8.37	------	.71	1.00	2.80	1.71
	Dry weight	5.63	12.56	25.15	15.72	37.71	------	4.63	6.81	18.42	11.44
Aug. 31	Fresh weight	1.56	3.97	6.83	3.25	10.80	.20	.98	.98	2.70	1.96
	Dry weight	8.98	22.83	31.44	14.97	54.27	1.29	6.22	6.15	18.35	12.37
Sept. 16	Fresh weight	1.62	4.46	8.28	3.72	12.74	.17	.54	.88	3.60	1.42
	Dry weight	5.40	17.22	32.00	14.40	49.22	.94	3.00	4.90	19.95	7.90
Sept. 29	Fresh weight	1.92	5.77	8.48	4.02	14.25	.11	.80	1.50	3.20	2.30
	Dry weight	7.91	23.69	34.90	16.55	58.59	.06	4.79	9.03	19.45	13.82
Oct. 15	Fresh weight	1.67	5.83	6.61	3.50	12.44	------	.95	.85	2.90	1.80
	Dry weight	9.26	32.22	29.22	15.50	61.44	------	10.45	5.62	19.17	16.07
Oct. 30	Fresh weight	1.00	7.20	5.22	2.93	12.32	------	------	------	------	------
	Dry weight	5.16	36.35	26.81	15.07	63.16	------	------	------	------	------

In the experiments reported herein no attempt was made to separate the true starch from the dextrin. The values for starch reported, therefore, represent the true starch together with the dextrinlike group of compounds which Bakke, Gaessler, and Loomis discuss.

Table 1 shows the data for total and reducing sugars, starch, acid-hydrolyzable and the readily available carbohydrates for undisturbed plants and plants cultivated 9 days after emergence (biweekly) throughout the growing season of 1936. In most cases dates of sampling are at exactly 2-week intervals.

SEASONAL TREND OF CARBOHYDRATES IN BINDWEED ROOTS

The seasonal trend of the sugars is represented graphically in figure 1. The total sugar content of the undisturbed plants gradually increased from April to the last of October. The reducing sugars in the

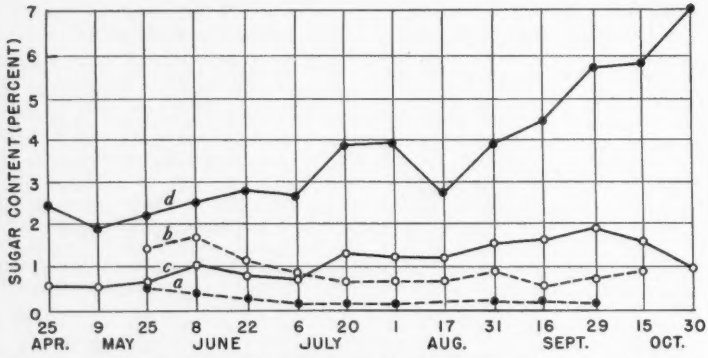

FIGURE 1.—Total and reducing sugar content of bindweed roots, when undisturbed and when cultivated at 2-week intervals, 1936: *a*, reducing sugars, cultivated; *b*, total sugars, cultivated; *c*, reducing sugars, undisturbed; *d*, total sugars, undisturbed.

undisturbed plants showed only slight increase from the beginning to the end of the experiment. Whether reducing sugars are precursors of sucrose or arise as a result of hydrolysis of the sucrose in bindweed is open to question. But in either case great variation in this fraction would not be expected, since in the former case they would rapidly be changed into the disaccharide and in the latter instance they would doubtless be used in general metabolism as rapidly as they were formed, without accumulating.

It has been shown in an earlier paper (*5*) that the total sugars of bindweed are materially depleted and are held at a minimum of about 1 percent of the dry weight by frequent cultivation. The data presented here show that when a cultivation program is started early in the spring the apparent depletion of total sugars is largely the result of a lack of normal accumulation rather than an actual depletion. Compare figure 1. Collection of samples from the cultivated plots was started May 25, 4 weeks after samples were first collected from the undisturbed plants. The total sugar content of the roots from the

cultivated plots was 0.75 percent less than from undisturbed plants. Slight depletion occurred, but the significant point seems to be the lack of accumulation in those plants which were repeatedly cultivated Only traces of reducing sugars were found in the roots of cultivated plants.

In the undisturbed series, the starch content showed greater variation than did the total sugars. The minimum values for both starch and total sugars on May 9 may be accounted for by the rapid utilization of carbohydrate material for regeneration and growth in the early spring. Observation of the growth status of the plants showed that four to six leaves had appeared on the young plants before May 5; about May 7 to 9 the plants exhibited much additional growth and marked vining. It seems reasonable to believe that this rapid vegetative growth would be a drain on the reserve foods since the limited photosynthetic area probably could not supply the high sugar requirements for rapid cell division.

The data show relatively more rapid accumulation of starch than sugar before October 1. The September 29 starch value was 471 percent of the value on April 25, while the total sugar for September 29 was about 236 percent of its value on the earlier date. The marked seasonal increase in percentage of starch as compared with the accumulation of total sugars may be interpreted to mean that while sucrose may act as a temporary storage product it is rapidly transformed into the more stable and insoluble polysaccharide material.

TABLE 2.—*Seasonal variations in the carbohydrate content (percent) of cultivated and of undisturbed bindweed roots expressed on fresh-weight and dry-weight bases, 1937*

Date	Basis	Undisturbed plants				Cultivated plants			
		Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 11	Fresh weight					0.95	1.70	3.10	2.65
	Dry weight					5.86	13.02	21.33	18.88
May 30	Fresh weight					1.59	1.74	3.14	3.33
	Dry weight					9.69	11.60	24.18	21.29
June 3	Fresh weight	1.94	1.74	3.76	3.68	2.42	2.44	2.10	4.86
	Dry weight	10.16	9.96	21.20	20.12	13.90	15.75	19.70	29.65
June 17	Fresh weight	1.97	2.83	3.28	4.80	2.15	3.60	3.09	5.75
	Dry weight	10.83	17.00	19.83	27.83	12.38	22.65	19.46	35.03
July 1	Fresh weight	1.97	2.84	3.44	4.81				
	Dry weight	9.96	15.70	21.36	25.66				
July 15	Fresh weight	1.70	3.13	3.07	4.83	1.96	4.05	2.43	6.01
	Dry weight	8.12	10.24	16.71	18.36	9.23	20.36	14.13	29.59
July 29	Fresh weight	3.08	7.50	2.45	10.58				
	Dry weight	12.67	33.82	10.72	46.49				
Aug. 12	Fresh weight	3.85	9.80	2.36	13.65	1.93	3.65	3.30	5.58
	Dry weight	16.90	41.90	10.70	58.80	9.11	17.63	14.15	26.74
Aug. 27	Fresh weight	3.78	8.84	2.91	12.62	3.11	3.88	3.22	6.99
	Dry weight	16.70	23.90	13.40	40.60	1.27	17.69	14.70	18.96
Sept. 11	Fresh weight	3.47	8.04	2.46	11.51	2.98	3.16	3.00	6.15
	Dry weight	13.05	35.10	10.47	48.15	14.14	16.75	15.85	30.89
Sept. 24	Fresh weight	2.75	7.47	3.38	10.22				
	Dry weight	11.14	32.10	15.27	43.24				
Oct. 7	Fresh weight	3.95	5.87	3.08	9.82				
	Dry weight	17.07	28.20	12.70	45.27				
Oct. 28	Fresh weight	5.00	4.63	2.73	9.63				
	Dry weight	22.62	25.30	15.34	47.92				

The change in percentage composition of sugar and starch in the roots of bindweed after about the end of September is interesting. Field notes taken indicate that temperatures had dropped considerably and growth had practically ceased near the end of September. Superficially the plants appeared to be dead and to be going into the usual winter dormant condition. It is a well-known fact that certain plants may be hardened to make them cold and drought resistant. Chemical analysis of such plants shows a marked change in the

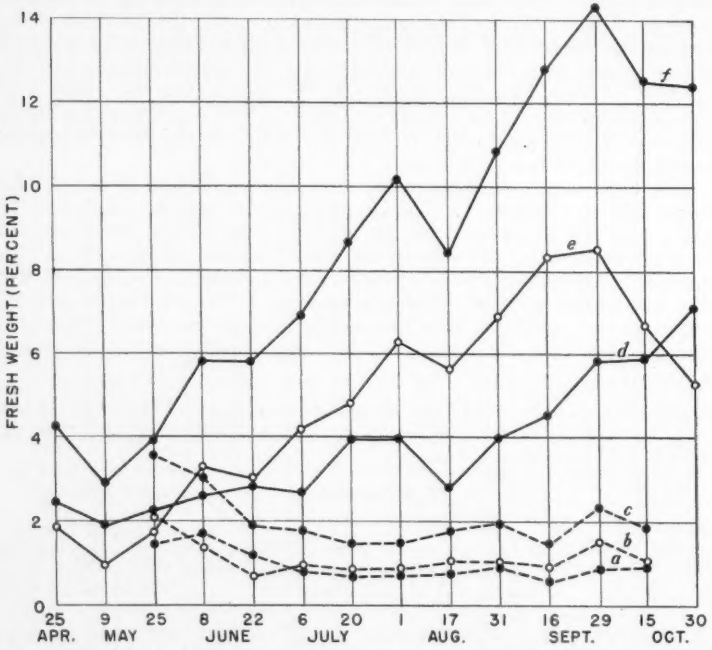

FIGURE 2.—Total sugar, starch, and readily available carbohydrate content of bindweed roots when undisturbed and when cultivated at 2-week intervals, 1936: *a*, Total sugars, cultivated; *b*, starch, cultivated; *c*, readily available carbohydrates, cultivated; *d*, total sugars, undisturbed; *e*, starch, undisturbed; *f*, readily available carbohydrates, undisturbed.

starch-sugar equilibrium. The marked decrease in starch content of bindweed roots accompanied by the increase in sugar percentage is indicative of the transition occurring in the roots with the approach of cold weather. Harvey (*12*) credits Lidforss (*16*) with reporting that a shift from polysaccharides to glucose and sucrose is a common occurrence in plants during the cold season. Hasselbring and Hawkins (*13*) have shown the same shift occurs in sweetpotatoes kept at low temperatures. Harvey (*12*) also has shown that the polysaccharides of hardened cabbages are displaced in the direction of monosaccharides and disaccharides.

Readily available reserves reached a maximum of 14.25 percent in the roots of undisturbed plants and a maximum of 3.51 percent in the cultivated plants.

Sugars, starch, and readily available carbohydrates, are plotted together in figure 2 to show their seasonal trends in undisturbed and cultivated plants. Distinct reductions are observed in each fraction A noteworthy fact which the graph reveals is the marked reduction in the carbohydrates of the cultivated plants.

Investigation of the seasonal trend of the carbohydrates was carried through the 1937 season also. The behavior of the carbohydrate

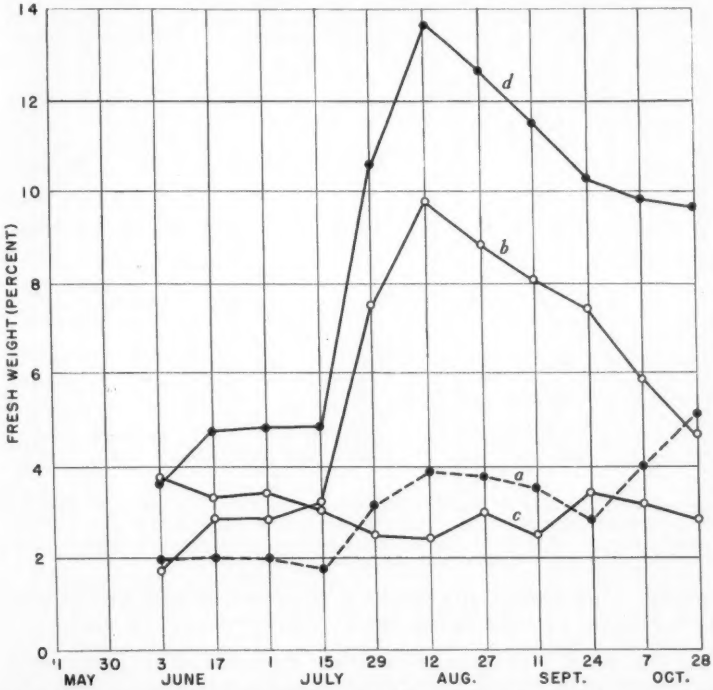

FIGURE 3.—Carbohydrate content of bindweed roots when undisturbed, 1937: *a*, Total sugars; *b*, starch; *c*, acid-hydrolyzable substances; *d*, readily available carbohydrates.

fractions in 1937 was similar to that in 1936 as shown in table 2 and figure 3. There was, however, more rapid accumulation of starch in the undisturbed plants. The data show that very little accumulation of starch occurred before July 15 but that thereafter there was a very rapid increase in the starch content, reaching a maximum of 9.80 percent by August 12th. This date was about 1 month earlier than the time of maximum carbohydrate accumulation in 1936. The data are shown in figure 4.

Weather data for 1937 are incomplete, but it is believed that the local environmental conditions delayed early accumulation and caused the rapid increase in starch between July 15 and August 12, in the undisturbed plants.

It should be pointed out that the samples used to show the seasonal trend of carbohydrates from fallowed plots for the 1937 series were taken from plots which were cultivated irregularly at 3- to 4-week intervals. This may account for the higher values found in the 1937 cultivated plants as compared with those of 1936.

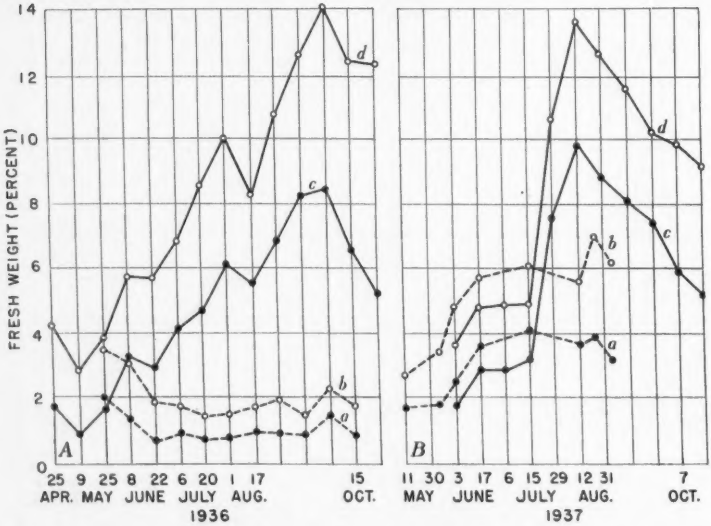

FIGURE 4.—Starch and readily available carbohydrate content of bindweed roots when undisturbed and when cultivated, 1936 (*A*) and 1937 (*B*): *a*, Starch, cultivated; *b*, readily available carbohydrates, cultivated; *c*, starch, undisturbed; *d*, readily available carbohydrates, undisturbed.

TREND OF CARBOHYDRATES IN BINDWEED ROOTS AFTER CROP HARVEST OR CULTIVATION

At the outset it was planned to study the trend of the food reserves in the roots of bindweed at short intervals of sampling after small-grain or other crop harvest and after one cultivation.

Assuming that rapid and important changes might be taking place in the carbohydrate fractions early in the season which could not be detected in the biweekly series, root samples were collected at 4-day intervals from May 2 to May 25, 1936. Analyses showed, however, that reducing sugars, total sugars and acid-hydrolyzable substances remained uniformly constant. The data are given in table 3. There was a moderate decline in the starch content from May 7 to 13 which was recovered by May 17, followed by gradual accumulation until May 25. During the period May 2 to May 25 the total sugar content was about 1 percent higher than the starch content.

Root samples of bindweed were collected at 4-day intervals for a period of 24 days after barley harvest. No unusual relationship was found between the several carbohydrate fractions (table 3), as a result

of the grain crop growing with the bindweed, nor did any striking changes take place after the barley was harvested.

TABLE 3.—*Variations in carbohydrate content (percent) of bindweed roots expressed on fresh-weight and dry-weight bases, 1936*

[Plants sampled at 4-day intervals May 2 to 25 and July 8 to August 1 (after barley harvest)]

MAY 2 TO 25

Date	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 2	Fresh weight	0.48	1.90	1.22	3.10	3.12
	Dry weight	3.01	11.87	7.60	19.32	19.47
May 7	Fresh weight	.65	2.19	1.36	3.05	3.55
	Dry weight	4.15	13.98	8.68	19.40	22.66
May 9	Fresh weight	.51	1.91	.93	3.10	2.84
	Dry weight	3.30	12.37	6.00	19.95	18.37
May 13	Fresh weight	.65	2.15	.90	2.80	3.05
	Dry weight	4.43	14.68	6.15	19.08	20.83
May 17	Fresh weight	.59	2.37	1.39	2.97	3.76
	Dry weight	3.75	15.00	8.78	18.78	23.78
May 21	Fresh weight	.60	2.28	1.55	2.71	3.83
	Dry weight	4.24	16.15	10.96	19.17	27.11
May 25	Fresh weight	.66	2.25	1.65	2.50	3.90
	Dry weight	4.48	15.30	11.28	16.92	26.58
JULY 8 TO AUGUST 1 (AFTER BARLEY HARVEST)						
July 8	Fresh weight	1.10	3.33	4.37	3.51	7.70
	Dry weight	5.80	17.63	21.03	16.87	38.66
July 12	Fresh weight	1.06	3.03	3.80	3.51	6.83
	Dry weight	5.39	15.43	19.43	17.92	34.86
July 16	Fresh weight	1.07	3.30	4.75	3.68	8.05
	Dry weight	.49	15.20	21.94	16.95	37.14
July 20	Fresh weight	1.54	4.28	4.74	3.24	9.02
	Dry weight	6.81	18.93	26.50	13.92	45.43
July 24	Fresh weight	2.32	5.14	7.71	3.49	12.85
	Dry weight	9.10	22.33	33.56	13.80	55.89
July 28	Fresh weight	2.14	5.14	7.90	3.85	13.04
	Dry weight	8.02	19.95	29.50	14.35	49.45
August 1	Fresh weight	1.23	3.96	6.22	3.50	10.18
	Dry weight	5.24	18.67	26.81	15.10	45.48

Two series of collections, usually at 4-day intervals, were made in 1937 in an effort to determine the extent of carbohydrate reduction after one cultivation. It was also believed that such a study would throw some light on the problem of carbohydrate relationships in regeneration.

In the first series the plot was cultivated May 4 and sampling was started May 11, upon emergence of the first plants. A marked decline occurred in both the sugar and starch content 4 days after emergence as compared with the values at emergence (table 4 and fig. 5). This decline was followed by a slight increase during the next 11 days. From May 11 to June 3 the starch was 0.2 to 0.8 percent higher than the total sugars. A closer relationship between these two fractions was shown here than at any other place in the study.

The first sample in the second series was collected July 29, when the plot was cultivated. Another sample was taken at emergence, and others (usually at 4-day intervals) thereafter for 24 days. Somewhat the same results were obtained at this time as earlier in the season. The total sugar content was practically the same as in the earlier series but did not show as much fluctuation. The starch content at cultivation was about 234 percent of the early spring value and dropped rapidly until 4 days after emergence and was still declining gradually at the end of the experiment.

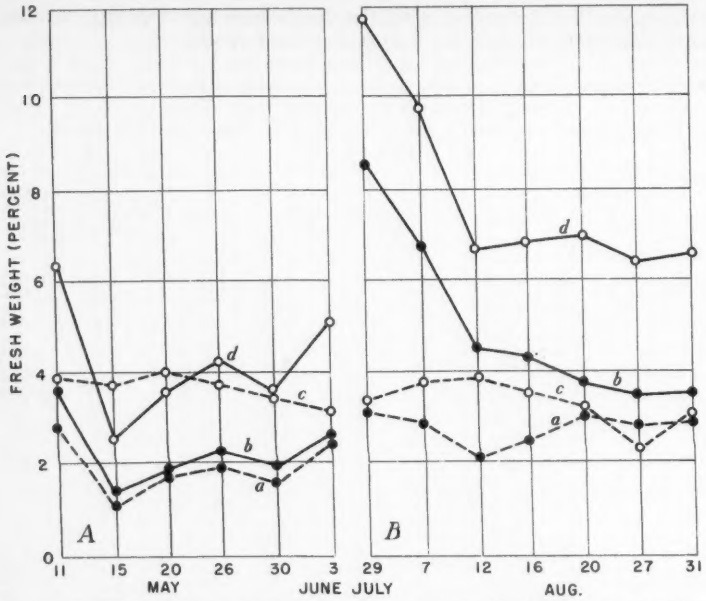

FIGURE 5.—Effect of one cultivation on the carbohydrate content of bindweed roots, 1937: *A*, Experiment performed in May; *B*, experiment performed in July. Sampled at emergence and at 4-day intervals for a period of 24 days: *a*, Total sugar; *b*, starch; *c*, acid-hydrolyzable substances; *d*, readily available carbohydrates.

TABLE 4.—*Carbohydrate content (percent) of bindweed roots after one cultivation expressed on fresh-weight and dry-weight bases, 1937*

MAY SERIES

Date	Basis	Total sugar	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
May 11	Fresh weight	2.78	3.61	3.93	6.39
	Dry weight	16.45	18.50	20.10	34.95
May 15	Fresh weight	1.11	1.43	3.79	2.54
	Dry weight	6.97	8.60	20.23	15.57
May 20	Fresh weight	1.72	1.92	4.06	3.64
	Dry weight	8.84	9.93	20.98	18.77
May 26	Fresh weight	1.91	2.33	3.76	4.24
	Dry weight	10.98	13.00	21.07	23.98
May 30	Fresh weight	1.59	1.91	3.43	3.50
	Dry weight	9.74	11.60	24.18	21.34
June 3	Fresh weight	2.42	2.67	3.17	5.09
	Dry weight	14.90	15.75	19.70	30.65

AUGUST SERIES

July 29	Fresh weight	3.15	8.48	3.42	11.63
	Dry weight	12.24	33.00	13.19	45 24
Aug. 7	Fresh weight	2.95	6.75	3.84	9.70
	Dry weight	12.06	22.62	15.73	34.68
Aug. 12	Fresh weight	2.11	4.58	3.94	6.69
	Dry weight	9.13	17.63	14.15	26.76
Aug. 16	Fresh weight	2.54	4.33	3.50	6.87
	Dry weight	11.45	20.10	17.01	31.55
Aug. 20	Fresh weight	3.11	3.87	3.22	6.98
	Dry weight	1.27	17.69	14.70	18.96
Aug. 27	Fresh weight	2.87	3.52	2.34	6.39
	Dry weight	11.08	32.10	11.75	43.18
Aug. 31	Fresh weight	2.98	3.53	3.13	6.51
	Dry weight	14.14	16.75	15.85	30.89

In general, the trend of the carbohydrates after a single cultivation showed an expected decline 4 to 10 days after cultivation with only moderate change for the rest of the 24-day period. It was concluded, therefore, that 6 intervals of 4 days each was not sufficient time for the plants to recover from the set-back brought about by the cultivation, and that subsequent investigation should be performed in a somewhat different fashion.

The information gathered from the carbohydrate studies of 1935 (*5*), 1936, and 1937 point also to the possibility of making total carbohydrate determinations, in certain studies at least, rather than attempting to fractionate the carbohydrates.

In 1938 the study of the carbohydrate trend after one cultivation was made in the following manner: Samples were collected but instead of being preserved in alcohol, they were dried by forced ventilation in an oven at 70° C. The dry material was then ground to proper fineness and hydrolyzed with 1+20 (i. e., 1 ml. of concentrated hydrochloric acid plus 20 ml. of water) hydrochloric acid at 120° for 1 hour, and determinations were made for reducing substances.

TABLE 5.—*Total carbohydrates (percent) by acid hydrolysis*[1] *in bindweed roots after one cultivation, expressed on fresh-weight and dry-weight bases, 1938*

Date	Total carbohydrates by acid hydrolysis on basis of—		Date	Total carbohydrates by acid hydrolysis on basis of—	
	Fresh weight	Dry weight		Fresh weight	Dry weight
June series			*August series*		
June 29	5.35	33.56	Aug. 5	13.05	43.62
July 6	2.82	30.25	Aug. 26	11.20	41.10
July 11	2.99	32.82	Sept. 6	9.84	38.20
July 19	3.08	30.75	Sept. 23	10.27	38.05
Aug. 3	3.42	32.20	Oct. 1	10.96	39.98
Aug. 9	3.52	33.90	Oct. 8	11.84	42.09

[1] Analysis for total carbohydrates made by acid hydrolysis of dry tissue and determinations made by the ceric sulfate method (*6*).

These data are presented in table 5. In the first series shown in figure 6 *A* the plot was sampled June 29 at the time of cultivation, again at the time of emergence of the bindweed, and at irregular intervals for about 1 month. The second series was started August 5. Cultivation was unavoidably delayed until August 15, and samples were collected at irregular intervals after emergence until October 8. The carbohydrate content at the beginning of the second series in August (Fig. 6 *B*) was 244 percent of that in June. Although a reduction of 47 percent was found in the total carbohydrate content from time of cultivation until emergence in the June series, the total reduction in the August series was but 25 percent. Depletion continued longer, however, at the later date, and the subsequent accumulation of total carbohydrates was relatively more rapid.

The difference in behavior of the reserve food in the two experiments might be explained in the following manner: A certain quantity of reserve food is used for the synthesis of new protoplasts. Removal of food from a low initial reserve caused a greater net effect than later in the season when the reserve was higher. The prolonged reduction after cultivation observed later in the season was probably owing to

less favorable growing conditions. The limited growth checked the demand for reserves, and as a consequence the photosynthate accumulated rapidly in spite of the limited photosynthetic area. This accumulation then aided materially in increasing the already relatively large supply of reserve materials in the roots.

NITROGEN VARIATIONS

Studies of the organic reserves in a large number of crop plants and noxious weeds have been made by Aldous (*1*), Arny (*2*), Barr (*5*), Graber et al (*9*), Grandfield (*10, 11*), McCarty (*19, 20*), Nelson (*24*),

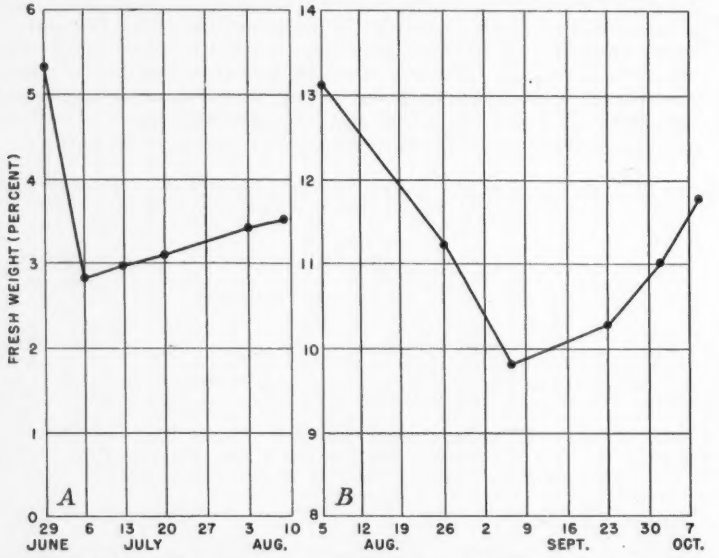

FIGURE 6.—Effect of one cultivation on the total carbohydrate content of bindweed roots, 1938: *A*, Experiment performed in June; *B*, experiment performed in August.

Pierre and Bertram (*25*), Welton, Morris, and Hartzler (*26*), and others. With few exceptions, attention has been directed chiefly to investigations of the carbohydrates. It is well known that the reserve carbohydrates are important factors in plant growth, but our knowledge relative to the several forms of nitrogen as they occur in storage organs of weeds is limited. It seemed, therefore, that a study of the various nitrogen fractions might throw some light on the problem of regeneration and growth in bindweed.

Analyses were made on the 1936 and 1937 root samples for nitrogen content. The dry material was analyzed by the Kjeldahl (*3*) method for colloidal or protein nitrogen. Determinations were made on the alcoholic extract for (1) ammonia and amide nitrogen according to Loomis and Shull (*18*), (2) amino nitrogen by the Van Slyke method, and (3) total nitrogen including nitrates by the modified Kjeldahl-salicylic acid method as described by Loomis and Shull (*18*). The data are given in table 6 and shown graphically in figure 7.

Table 6 and figure 7 show gradual but marked accumulation of colloidal nitrogen in the undisturbed plants from April to October. Cultivation held the colloidal nitrogen around 0.25 percent with very

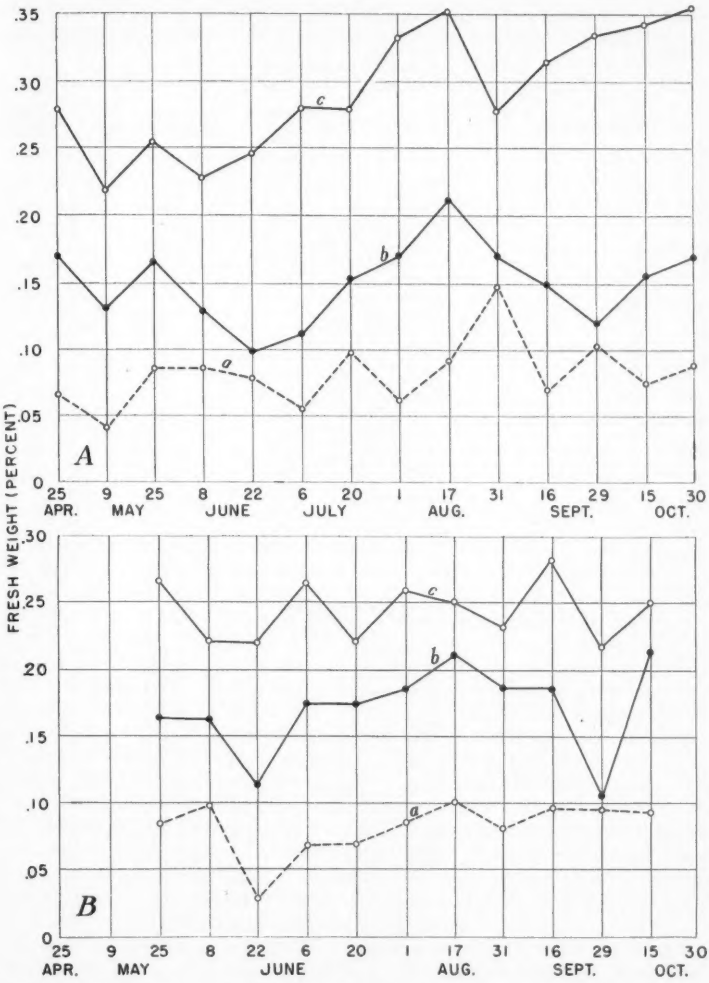

FIGURE 7.—Nitrogen content of bindweed roots when undisturbed (*A*) and when cultivated at 2-week intervals (*B*), 1936: *a*, Amino nitrogen; *b*, soluble nitrogen; *c*, colloidal nitrogen.

little change throughout the season. The data show that cultivation reduced the colloidal-nitrogen content, although frequency of cultivation appeared to have no material effect. No important changes occurred in the soluble-nitrogen content in either cultivated or undis-

turbed plants. Traces of amide nitrogen and moderate quantities of nitrates and nitrites which showed no consistent variations were found.

TABLE 6.—*Seasonal variations in nitrogen content (percent) of bindweed roots, when undisturbed and when cultivated, expressed on fresh weight and dry weight bases, 1936–37*

UNDISTURBED, 1936

Series and date	Basis	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
Apr. 25	Fresh weight	0.27	0.17	0.067
	Dry weight	1.67	1.04	.40
May 9	Fresh weight	.21	.13	.041
	Dry weight	1.40	.84	.26
May 25	Fresh weight	.25	.16	.086
	Dry weight	1.72	1.14	.29
June 8	Fresh weight	.22	.12	.082
	Dry weight	1.51	.85	.54
June 22	Fresh weight	.24	.09	.076
	Dry weight	1.44	.79	.31
July 6	Fresh weight	.28	.11	.056
	Dry weight	1.51	.59	.29
July 20	Fresh weight	.27	.15	.098
	Dry weight	.94	.65	.43
Aug. 1	Fresh weight	.33	.17	.063
	Dry weight	1.45	.76	.13
Aug. 17	Fresh weight	.35	.21	.087
	Dry weight	1.58	.94	.39
Aug. 31	Fresh weight	.27	.17	.148
	Dry weight	1.27	.81	.68
Sept. 16	Fresh weight	.31	.15	.069
	Dry weight	1.21	.61	.34
Sept. 29	Fresh weight	.33	.12	.102
	Dry weight	1.36	.32	.42
Oct. 15	Fresh weight	.34	.15	.071
	Dry weight	1.54	.67	.31
Oct. 30	Fresh weight	.35	.17	.082
	Dry weight	1.80	.87	.45

CULTIVATED, 1936

May 25	Fresh weight	0.26	0.16	0.082
	Dry weight	1.53	.98	.48
June 8	Fresh weight	.22	.16	.098
	Dry weight	1.37	1.00	.60
June 22	Fresh weight	.21	.11	.027
	Dry weight	1.27	.65	.15
July 6	Fresh weight	.26	.17	.068
	Dry weight	1.61	1.05	.41
July 20	Fresh weight	.22	.17	.071
	Dry weight	1.48	.59	.33
Aug. 1	Fresh weight	.25	.18	.085
	Dry weight	1.56	1.10	.51
Aug. 17	Fresh weight	.24	.21	.100
	Dry weight	1.63	1.41	.65
Aug. 31	Fresh weight	.22	.18	.083
	Dry weight	1.45	1.07	.52
Sept. 16	Fresh weight	.27	.18	.095
	Dry weight	1.54	1.02	.52
Sept. 29	Fresh weight	.21	.10	.094
	Dry weight	1.30	.60	.28
Oct. 15	Fresh weight	.24	.21	.090
	Dry weight	1.64	1.38	.59

TABLE 6.—*Seasonal variations in nitrogen content (percent) of bindweed roots, when undisturbed and when cultivated, expressed on fresh weight and dry weight bases, 1936–37*—Continued

AFTER ONE CULTIVATION, 1937

Series and date	Basis	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
First series:				
May 11	Fresh weight	0.25	0.26	0.089
	Dry weight	.15	1.57	.52
May 15	Fresh weight	.18	.16	.042
	Dry weight	1.16	1.07	.26
May 20	Fresh weight	.25	.25	.077
	Dry weight	1.30	1.29	.39
May 26	Fresh weight	.19	.18	.054
	Dry weight	1.14	1.07	.31
May 30	Fresh weight	.19	.15	.050
	Dry weight	1.16	.97	.30
June 3	Fresh weight	.16	.10	.053
	Dry weight	.95	.60	.31
Second series:				
July 29	Fresh weight	.27	.18	.082
	Dry weight	1.03	.68	.31
Aug. 7	Fresh weight	.22	.14	.057
	Dry weight	.91	.60	.23
Aug. 12	Fresh weight	.21	.10	.051
	Dry weight	.92	.46	.22
Aug. 16	Fresh weight	.19	.11	.046
	Dry weight	.86	.50	.10
Aug. 20	Fresh weight	.21	.14	.049
	Dry weight	.90	.57	.20
Aug. 27	Fresh weight	.20	.10	.046
	Dry weight	.89	.45	.21
Aug. 31	Fresh weight	.19	.12	.062
	Dry weight	.92	.57	.29

The most significant fluctuations were found in the roots collected at 4-day intervals after one cultivation. The soluble nitrogen content of the early-season (first) series (table 6 and fig. 8) was relatively high, having a value at the time of cultivation slightly above the colloidal nitrogen in the residue. The marked drop in both colloidal and soluble nitrogen is important and can be accounted for by the rapid utilization of nitrogenous compounds in the quick growth of the plants at that time of the year. The plot from which these samples were collected was cultivated on May 4 and showed profuse emergence on May 11 when the first sample of the series was collected. The marked fluctuation in percentage of colloidal nitrogen and total soluble nitrogen from May 11 to 20 is not understood. It seems quite probable that the initiation of new shoot growth would stimulate the digestion of stored proteins and thus reduce the percentage of these materials in the root tissue. Loomis (*17*) has shown that in woody plants initiation of shoot growth is correlated with a relatively high concentration of organic nitrogen compounds.

If soluble organic nitrogenous compounds are related to shoot growth in bindweed, the transition of stored proteins to soluble nitrogen would account for the rapid drop in protein after cultivation. Continued reduction of protein nitrogen for the next 10 to 15 days would be expected since considerable vegetative growth occurred during that period. This behavior is also in harmony with the trends of the sugar and starch which suggest that photosynthesis after the new plants were well established was adequate to supply the carbohydrate needs for regeneration but not sufficient to allow for any accumulation.

THE EFFECT OF SODIUM CHLORATE ON THE ORGANIC RESERVES IN BINDWEED

Observations made by a number of investigators (*8, 14, 15, 21, 23, 27*) have shown that sodium chlorate is generally more effective as a weed killer when applied late in the summer and fall than at any time earlier in the growing season. Unpublished results of experiments at this station with whiteweed suggested the possibility that the effect of sodium chlorate could be increased by making applications subsequent to cultivation. It was assumed that the stimulation of physiological activities by cultivation would have an initial effect of reducing the reserve food. If, then, sodium chlorate were applied, the net result

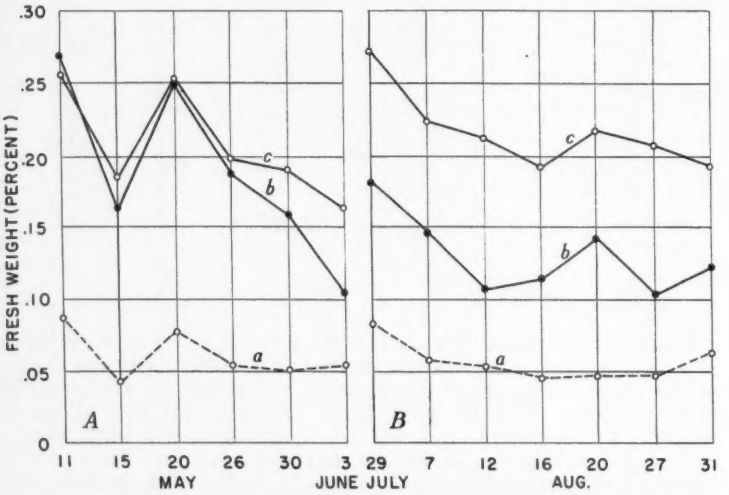

FIGURE 8.—Effect of one cultivation on the nitrogen content of bindweed roots 1937: *A*, Experiment performed in May; *B*, experiment performed in July: *a* Amino nitrogen; *b*, soluble nitrogen; *c*, colloidal nitrogen.

should be greater than either treatment alone. It was further believed that the application of sodium chlorate previous to cultivation probably caused the plant to become temporarily dormant which accounted for the observation that apparently better results were obtained by the addition of sodium chlorate subsequent to cultivation.

It seemed, therefore, that experiments designed to study the root reserves as affected by sodium chlorate applied to cultivated plants at various dates through the year might yield results which would be of value in developing a plant of control.

Several plots were selected which were uniformly infested with bindweed. A series of treatments was arranged so that sodium chlorate was applied to one plot June 1; another plot was treated July 1; another August 1, and so on until the middle of September. A similar series of chlorate treatments was made on plots cultivated at weekly intervals during the same period. Samples were taken when the chlorate was applied and also 1 month after its application. This arrangement provided a plan for the study of the root reserves as

affected by sodium chlorate when applied at various dates during the season and also of the effect of a combined treatment of chlorate and cultivation.

Results of analyses showed that the carbohydrate content was held to a lower value by cultivation alone than by application of sodium chlorate alone. The data of tables 7 and 8 show also that sodium

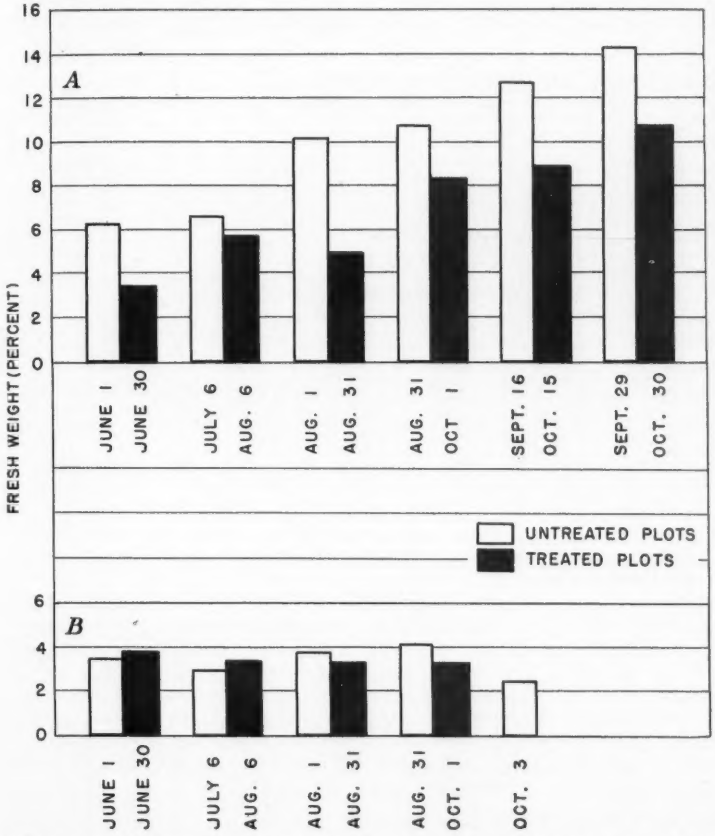

FIG. 9.—Carbohydrate content of bindweed roots as affected by date of application of sodium chlorate to undisturbed and to cultivated plots, 1936: *A*, Undisturbed; *B*, cultivated.

chlorate was relatively less effective (in reducing the carbohydrate reserve) on cultivated plants than on those which were undisturbed. The data are shown graphically in the histogram of figure 9. It may be concluded from these data that the application of sodium chlorate to bindweed under clean cultivation resulted in no important reduction of carbohydrate content beyond the decline obtained by clean cultivation.

TABLE 7.—*Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content percent of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936*

Date	Basis	Undisturbed plants		Cultivated plants	
		Total carbohydrates	Readily available carbohydrates	Total carbohydrates	Readily available carbohydrates
SAMPLED AT TIME OF TREATMENT					
June 1	Fresh weight	9.46	6.06	6.45	3.55
	Dry weight	54.34	34.96	40.57	21.87
July 6	Fresh weight	9.76	6.56	5.27	2.87
	Dry weight	51.39	34.81	35.46	19.01
Aug. 1	Fresh weight	13.66	10.16	6.13	3.73
	Dry weight	60.58	45.48	43.52	26.22
Aug. 31	Fresh weight	13.97	10.77	7.25	4.15
	Dry weight	69.29	54.31	42.36	24.08
Sept. 16	Fresh weight	16.46	12.76		
	Dry weight	63.62	49.22		
Sept. 29	Fresh weight	18.27	14.27		
	Dry weight	75.14	58.59		
Oct. 30	Fresh weight			5.87	2.32
	Dry weight			35.22	13.92
SAMPLED 1 MONTH AFTER TREATMENT					
June 30	Fresh weight	6.52	3.32	6.44	3.74
	Dry weight	39.71	20.76	42.94	24.39
Aug. 6	Fresh weight	8.64	5.74	5.48	3.38
	Dry weight	52.02	34.47	45.31	28.06
Aug. 31	Fresh weight	8.54	4.94	5.52	3.12
	Dry weight	41.99	24.41	46.55	30.63
Oct. 1	Fresh weight	11.55	8.25	5.81	3.01
	Dry weight	55.46	39.69	38.94	20.12
Oct. 15	Fresh weight	12.52	8.82		
	Dry weight	59.01	42.06		
Oct. 30	Fresh weight	13.52	10.82		
	Dry weight	73.05	58.15		

TABLE 8.—*Effect of combined treatment with sodium chlorate and cultivation on the nitrogen content (percent) of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936*

Date	Basis	Undisturbed plants		Cultivated plants	
		Colloidal nitrogen	Soluble nitrogen	Colloidal nitrogen	Soluble nitrogen
SAMPLED AT TIME OF TREATMENT					
June 1	Fresh weight	0.21	0.20	0.23	0.15
	Dry weight	1.24	.57	1.48	.94
July 6	Fresh weight	.28	.11	.21	.21
	Dry weight	1.51	.59	1.45	1.44
Aug. 1	Fresh weight	.33	.17	.21	.15
	Dry weight	1.45	.76	1.48	1.13
Aug. 31	Fresh weight	.22	.15	.21	.14
	Dry weight	1.26	.71	1.25	.86
Sept. 16	Fresh weight	.31	.15		
	Dry weight	1.21	.61		
Sept. 29	Fresh weight	.33	.07		
	Dry weight	1.36	.32		
Oct. 3	Fresh weight			.24	.16
	Dry weight			1.54	1.00
SAMPLED 1 MONTH AFTER TREATMENT					
June 30	Fresh weight	0.27	0.28	0.23	0.52
	Dry weight	1.61	1.65	1.56	3.74
Aug. 6	Fresh weight	.27	.16	.17	.14
	Dry weight	1.61	.96	1.35	1.19
Aug. 31	Fresh weight	.31	.16	.20	.17
	Dry weight	1.84	.82	1.31	1.12
Oct. 1	Fresh weight	.37	.20	.20	.08
	Dry weight	1.76	.97	1.34	.58
Oct. 15	Fresh weight	.39	.19		
	Dry weight	1.80	.89		
Oct. 30	Fresh weight	.33	.11		
	Dry weight	1.74	.61		

The general trend of the organic reserves in the chlorated and cultivated plants was essentially the same for 1937 as for 1936.

Estimated regrowth based on observations made in June 1939 indicated that from 50- to 75-percent eradication was obtained on uncultivated plots by the application of sodium chlorate in June, July, or August 1938 while 88- to 92-percent eradication was obtained by the September treatments. Approximately 65- to 75-percent eradication was obtained by the application of sodium chlorate in June, July, or August to cultivated plots, and 95 to 97-percent when applied in September.

TABLE 9.—*Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content (percent) of bindweed roots, expressed on fresh-weight and dry-weight, 1938.*[1]

Date of cultivation and chlorate treatment		Date of sampling	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates
Cultivated	Treated							
May 10 and June 7	May 10	June 10	Fresh weight	0.54	1.20	0.61	3.18	1.81
	do		Dry weight	3.83	8.40	4.31	22.41	12.71
None	do		Fresh weight	.59	1.69	1.03	3.43	2.74
			Dry weight	3.97	11.34	7.00	22.93	18.34
May 10 and June 7	June 10	July 10	Fresh weight	1.17	2.42	.89	3.67	3.31
	do		Dry weight	7.27	15.03	5.56	22.80	20.59
None	do		Fresh weight	.52	1.09	1.04	3.77	2.13
			Dry weight	3.25	6.84	6.50	23.60	13.34
May 10 and June 7	July 9	Oct 15	Fresh weight	2.23	10.53	2.01	3.46	12.54
	do		Dry weight	10.14	47.66	9.12	15.70	56.78
None	do		Fresh weight	2.60	5.00	3.18	3.28	8.18
			Dry weight	11.90	27.98	14.53	14.95	42.51
May 10 and June 7	Aug. 5	Oct 22	Fresh weight	1.86	8.64	2.59	3.22	11.23
	do		Dry weight	7.54	40.44	12.15	15.07	52.59
None	do		Fresh weight	2.14	5.75	1.81	2.99	7.56
			Dry weight	11.31	30.29	9.62	15.82	39.91
May 10 and June 7	Sept. 23		Fresh weight	.83	5.24	1.11	2.73	6.35
	do		Dry weight	4.18	26.43	5.59	13.75	32.02
None	do		Fresh weight	.96	6.88	.57	1.99	7.45
			Dry weight	5.49	39.31	3.28	11.40	42.59

[1] Carbohydrates determined by use of ceric sulfate (*6*). Samples were taken at the time of treatment and 1 month after treatment for the first 2 months; no samples were taken following the July, August, and September treatments until the end of the season in October.

Table 9 shows the reducing sugars, total sugars, starch, and readily available carbohydrates. It should be noted that samples were taken at the time of treatments and 1 month after treatment for the first 2 months, while no samples were taken following the July, August, and September treatments until the end of the season, in October.

Results of experiments for the 3-year period 1936–38 consistently showed no important reduction of carbohydrates when attempts were made to weaken the plants by pretreatments of cultivation before applying chlorates. This is in accord with the observations made by Crafts (*3*) on resprouting of bindweed after hoeing and various herbicidal spray treatments.

Analysis of the dry residue for total nitrogen and the alcoholic extract for total soluble and amino nitrogen failed to give results indicating that nitrogen was significantly affected by a combined chlorate and fallow treatment.

RESERVES IN BINDWEED ROOTS AT END OF SEASON

In table 10 and figure 10 are shown reducing sugar, total sugar, starch and acid hydrolyzable values of bindweed roots collected at the end of the 1936 growing season from plots which were (1) undisturbed.

(2) cultivated all season at emergence, and (3) cultivated 3, 6, 9, 12, and 15 days after emergence. The starch content of undisturbed plants was over 700 percent of that of plants cultivated at emergence. The sugar content on the other hand in the undisturbed plants was only 360 percent of that found in the plants cultivated at emergence. Cultivation reduced the acid-hydrolyzable substances and hexose sugars only moderately, as would be expected since the acid-hydrolyzable materials do not constitute a readily available reserve and hexose sugars are present only in small quantities. Although more frequent

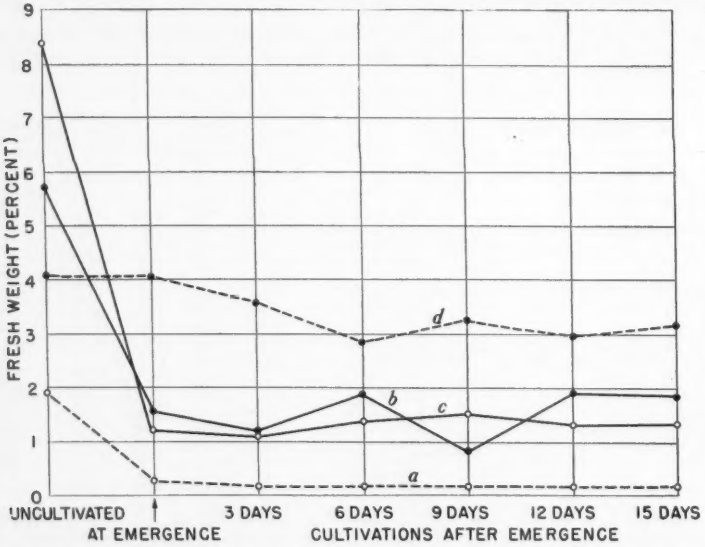

FIG. 10.—Carbohydrate content of bindweed roots when undisturbed and when cultivated at emergence and 3, 6, 9, 12, and 15 days after emergence, 1936, sampled in October: *a*, Reducing sugars; *b*, total sugars; *c*, starch; *d*, acid-hydrolyzable substances.

cultivation aided materially in diminishing the regeneration and persistence of the weed, it seemed to have no practical advantage over the less frequent cultivation (6, 9, 12, or 15 days after emergence) in reducing the values of the various carbohydrate fractions. These results are in close agreement with those reported in an earlier paper (*5*). The carbohydrate content of the roots of plants cultivated to a depth of 6 inches showed no important variation from that of plants cultivated to the depth of 3 inches. Data on this problem are meager, but it would seem that the advantage of deep cultivation for the purpose of reducing root reserves is open to question.

The clean-cultivation program started in 1936 was continued through 1937 and 1938. Emergence of the plants was somewhat retarded, and the time between cultivations increased for all the tests.

By October 1937 only a few scattered plants continued to emerge, and the quantity of roots which could be collected was so small that in some cases analyses could not be made. A small area of bindweed persisted, however, covering portions of two adjoining plots well into

the 1938 season after 3 years of clean cultivation at intervals of 6 and 9 days after emergence. Roots were collected, of which the analyses showed that the total sugar content was about 2.5 percent and starch about 1 percent.

TABLE 10.—*Effect of cultivation on carbohydrate and nitrogen content (percent) of bindweed roots, expressed on fresh-weight and dry-weight bases, 1936*

When cultivated	Basis	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates	Colloidal nitrogen	Total soluble nitrogen	Amino nitrogen
Undisturbed	Fresh weight	1.92	5.77	8.48	4.02	14.25	0.33	0.12	0.102
	Dry weight	7.91	23.69	34.90	16.55	58.59	1.36	.32	.42
At emergence (3 inches)	Fresh weight	.27	1.59	1.19	4.02	2.78	.21	.21	.026
	Dry weight	1.56	9.26	6.90	23.35	16.16	1.27	1.26	.15
3 days after emergence	Fresh weight	.14	1.17	1.15	3.55	2.32	.24	.14	.059
	Dry weight	.86	7.02	6.90	21.30	13.92	1.49	.85	.35
6 days after emergence	Fresh weight	.20	1.89	1.36	2.81	3.25	.19	.14	.085
	Dry weight	1.60	15.23	10.90	19.40	26.13	1.52	.57	.49
9 days after emergence	Fresh weight	.11	.80	1.50	3.24	2.30	.21	.10	.094
	Dry weight	.06	4.79	9.03	19.45	13.82	1.30	.60	.28
12 days after emergence	Fresh weight	.10	1.88	1.27	2.92	3.15	.25	.11	.083
	Dry weight	1.36	25.86	7.00	16.00	32.86	1.38	.65	.45
15 days after emergence	Fresh weight	.10	1.85	1.27	3.12	3.12	.19	.10	.054
	Dry weight	.77	11.50	7.94	19.42	19.44	1.18	.67	.33
9 days after emergence (6 inches).	Fresh weight	.03	1.40	1.05	3.90	2.45	.20	.11	.073
	Dry weight	.17	8.25	5.62	20.80	13.87	1.06	.62	.38

The relatively high carbohydrate content clearly indicates movement of food from the deeper roots, since continued clean cultivation for the 2-year period had allowed no photosynthesis to take place. The results obtained here are not to be confused or, perhaps, even compared with results ordinarily obtained, since this is a single exceptional case of persistent regrowth. The behavior of the plants on this small area was very unusual and unexpected even after one season of clean cultivation. No explanation other than a possible varietal difference or peculiar soil condition can be offered to account for the persistence of the plants on the area in question.

SUMMARY

The carbohydrate and the nitrogen content of the roots of bindweed (*Convolvulus arvensis* L.) as affected by various cultural methods and chemical treatments were studied, and the results of experiments covering 3 years' investigation are reported. Data are presented to show the seasonal trend of the reducing sugars, total sugars, starch, and acid-hydrolyzable substances, for undisturbed plants and for those cultivated 9 days after emergence. The maximum reducing-sugar content did not exceed about 2 percent of the fresh weight of the roots of undisturbed plants. Total sugars reached a maximum of about 7 percent in late October, with a minimum value in May.

The percentage of starch increased gradually, reaching a maximum in August or September, followed by a sharp decline conicident with a rapid increase in percentage of total sugar. This behavior suggests the shift of polysaccharides to soluble carbohydrates which commonly occurs in plants during the cold season. Cultivation of the plants at 2-week intervals held the total sugar and starch content each to about 1 percent. The readily available carbohydrate accumulation in the roots of cultivated plants was reduced to 2.30 percent, which was 16 percent of that of undisturbed plants.

One cultivation the first part of May 1937 reduced the sugar and the starch content to about 2 percent each, while cultivation the latter part of July reduced the starch content almost 50 percent without any important effect on the total sugars. Similar experiments in 1938 showed comparable results, indicating the advantage of early fallowing.

The colloidal nitrogen in the roots of the undisturbed plants showed gradual increase from May until the last of October. Cultivation checked this accumulation. The total soluble, amino, amide, nitrate, and nitrite nitrogen fractions showed no consistent variations. On the basis of the results obtained for total nitrogen and nitrogen fraction determinations it would appear that the importance of nitrogen in a study of root reserves in bindweed is open to question.

The carbohydate content was held to a lower value by cultivation than by application of sodium chlorate.

A combined treatment of sodium chlorate following early-season cultivation did not increase the effectiveness of the chlorate in reducing the carbhoydrate reserve.

Fall applications of sodium chlorate to uncultivated plants were relatively more effective than early applications in reducing the root reserves and in controlling the weed.

Plants cultivated systematically all season at emergence, and 3, 6, 9, 12, or 15 days after emergence were uniformly low in starch content, 1 to 2 percent, as determined by taka-diastase digestion. The data showed that cultivating at emergence had essentially no greater effect than the less frequent cultivations in reducing the carbohydrate content of bindweed roots in the top foot of soil.

LITERATURE CITED

(1) Aldous, A. E.
1930. relation of organic food reserves to the growth of some kansas pasture plants. Amer. Soc. Agron. Jour. 22: 385–392.

(2) Arny, A. C.
1932. variations in the organic reserves in underground parts of five perennial weeds from late april to november. Minn. Agr. Expt. Sta. Tech. Bul. 84, 28 pp., illus.

(3) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis . . . Ed. 4, 710 pp., illus. Washington, D. C.

(4) Bakke, A. L., Gaessler, W. G., and Loomis, W. E.
1939. relation of root reserves to control of european bindweed, convolvulus arvensis l. Iowa Agr. Expt. Sta. Res. Bul. 254, pp. 115–144, illus.

(5) Barr, C. Guinn.
1936. preliminary studies on the carbohydrates in the roots of bindweed. Amer. Soc. Agron. Jour. 28: 787–798, illus.

(6) ———
1939. application of the ceric sulphate method in the analysis of carbohydrates in the roots of lepidium and convolvulus. Plant Physiol. 14: 285–296, illus.

(7) Bertrand, Gabriel.
1906. le dosage des sucres réducteurs. Soc. Chim. de Paris Bul. 35: 1285–1299.

(8) Crafts, A. S.
1935. factors influencing the effectiveness of sodium chlorate as a herbicide. Hilgardia 9: 437–457.

(9) Graber, L. F., Nelson, N. T., Leukel, W. A., and Albert, W. B.
1927. organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wis. Agr. Expt. Sta. Res. Bul. 80, 128 pp., illus.

(10) GRANDFIELD, C. O.
1930. THE RELATION OF ORGANIC FOOD RESERVES TO THE EFFECT OF CUTTING PASTURE WEEDS AT DIFFERENT STAGES OF GROWTH. Amer. Soc. Agron. Jour. 22: 709–713, illus.

(11) ———
1935. THE TREND OF ORGANIC FOOD RESERVES IN ALFALFA ROOTS AS AFFECTED BY CUTTING PRACTICES. Jour. Agr. Res. 50: 697–709, illus.

(12) HARVEY, R. B.
1918. HARDENING PROCESS IN PLANTS AND DEVELOPMENT FROM FROST INJURY. Jour. Agr. Res. 15: 83–111, illus.

(13) HASSELBRING, HEINRICH, and HAWKINS, LON A.
1915. CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES. Jour. Agr. Res. 5: 543–560.

(14) HULBERT, H. W., BRISTOL, R. S., and BENJAMIN, L. V.
1931. METHODS AFFECTING THE EFFICIENCY OF CHLORATE WEED KILLERS. Idaho Agr. Expt. Sta. Bul. 189, 12 pp.

(15) KIESSELBACH, T. A., PETERSEN, N. F., and BURR, W. W.
1934. BINDWEEDS AND THEIR CONTROL. Nebr. Agr. Expt. Sta. Bul. 287, 47 pp., illus.

(16) LIDFORSS, B.
1909. DIE WINTERGRÜNE FLORA. EINE BIOLOGISCHE UNTERSUCHUNG. Lunds Univ. Årsskr., N. F. bd. 2, Afd. 2, No. 13, 76 pp., illus. [Original not seen. Abstract in Bot. Centbl. 110: 291–293.]

(17) LOOMIS, W. E.
1935. TRANSLOCATION AND GROWTH BALANCE IN WOODY PLANTS. Ann. Bot. [London] 49: [247]–272, illus.

(18) LOOMIS, WALTER E., and SHULL, CHARLES A.
1937. METHODS IN PLANT PHYSIOLOGY; A LABORATORY MANUAL AND RESEARCH HANDBOOK. 472 pp., illus. New York and London.

(19) MCCARTY, EDWARD C.
1935. SEASONAL MARCH OF CARBOHYDRATES IN ELYMUS AMBIGUUS AND MUHLENBERGIA GRACILIS, AND THEIR REACTION UNDER MODERATE GRAZING USE. Plant Physiol. 10: 727–738, illus.

(20) ———
1938. THE RELATION OF GROWTH TO THE VARYING CARBOHYDRATE CONTENT IN MOUNTAIN BROME. U. S. Dept. Agr. Tech. Bul. 598, 24 pp., illus.

(21) MUENCHER, W. C.
1932. KILLING PERENNIAL WEEDS WITH CHLORATES DURING WINTER. N. Y. (Cornell) Agr. Expt. Sta. Bul. 542, 8 pp., illus.

(22) MUNSON, L. D., and WALKER, P. H.
1906. THE UNIFICATION OF REDUCING SUGAR METHODS. Amer. Chem. Soc. Jour. 28: 663–686.

(23) NELLER, J. R.
1931. EFFECT OF CHLORATES UPON THE CATALASE ACTIVITY OF THE ROOTS OF BINDWEED. Jour. Agr. Res. 43: 183–189, illus.

(24) NELSON, N. T.
1925. THE EFFECTS OF FREQUENT CUTTING ON THE PRODUCTION, ROOT RESERVES, AND THE BEHAVIOR OF ALFALFA. Amer. Soc. Agron. Jour. 17: 100–113.

(25) PIERRE, W. H., and BERTRAM, F. E.
1929. KUDZU PRODUCTION WITH SPECIAL REFERENCE TO FREQUENCY OF CUTTING ON YIELDS AND FORMATION OF ROOT RESERVES. Amer. Soc. Agron. Jour. 21: 1079–1101, illus.

(26) WELTON, F. A., MORRIS, V. H., and HARTZLER, A. J.
1929. ORGANIC FOOD RESERVES IN RELATION TO THE ERADICATION OF CANADA THISTLES. Ohio Agr. Expt. Sta. Bul. 441, 25 pp., illus.

(27) ZAHNLEY, J. W., and PICKETT, W. F.
1934. FIELD BINDWEED AND METHODS OF CONTROL. Kans. Agr. Expt. Sta. Bul. 269, 26 pp., illus.

DIAMETER RELATIONSHIPS OF WOOL FIBERS FROM FIVE BREEDS OF SHEEP RAISED IN SOUTH DAKOTA [1]

By BARBARA BAILEY [2]

Research assistant in home economics, South Dakota Agricultural Experiment Station

INTRODUCTION

Average fiber diameter, its variability, and fiber contour are important factors in the grading of wool fiber and fleeces, inasmuch as the present wool-grading system is based on fiber diameter. While average diameter may indicate, in general, the grade of wool, the calculation of the deviation from the average is required to evince uniformity of fleece or sample. The third factor mentioned, fiber contour, has been pointed out by Barker (*3, pp. 169–179*) [3] as influencing the spinning qualities of wool fiber.

As part of a study of fabrics manufactured from wool from five breeds of sheep raised at the South Dakota Agricultural Experiment Station, fiber studies have been conducted on samples from three locations on each fleece. The results of these studies are reported herein. The details of sampling are given in the section on methods and materials.

REVIEW OF LITERATURE

Measurements of fiber diameter have been made by three methods: The weight-per-unit-length, the caliper, and the optical method. Krauter (*10*) in a paper published in 1929 presented an extensive review of the methods which had been used up to that time. Roberts (*12*) discussed in detail the weight-per-unit-length method which depends upon the fact that wool fiber practically always has the same specific gravity. Burns and Koehler (*6*) and later Burns (*5*) described the use of micrometer calipers for determining fiber diameter.

The optical methods which are now generally employed are of two types. The first, which was developed by the Wool Industries Research Association in England, is now recognized as official by the International Wool Textile Organization. In this case a small number of fibers are cut into very short lengths throughout the entire length of the fibers. Thus the measurement takes into account any variation in diameter that may exist along the length of the fibers. This method has been described in detail, and its applications in the study of various British breeds of sheep are reported by Wildman (*17*), Stanbury and Daniels (*16*), and Wildman and Daniels (*18*).

The second method, which is being widely used in the United States, is the so-called cross-section method and is recognized as an official method by the American Society for Testing Materials (*2*). Sidey (*14*)

[1] Received for publication May 18, 1939. Journal Series 113, of the South Dakota Agricultural Experiment Station, Brookings, S. Dak.

[2] The author wishes to express her gratitude to Dr. F. R. Immer, professor of agronomy and plant genetics, University of Minnesota, for his assistance with the statistical analyses of the data; to Barbara Webster, former student at South Dakota State College, who prepared the microscopic slides; and to Dr. S. P. Swenson, associate professor of agronomy, South Dakota State College, for reviewing the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 425.

reported a cross-section study of New Zealand Romney and Corriedale wools in 1931. In this case the fibers were embedded in paraffin and a microtome was used for preparing the cross-section slides. Recently rapid cross-sectioning devices have been described by Hardy (*1*, *8*) and by Schwarz (*13*). The cross-section procedure for diameter measurements has the advantage over the International Wool Textile Organization method in that it is possible to determine fiber contour in the same operation. This advantage becomes of particular importance in breeding work where it is desired to select animals which approach the ideal ratio of 1:1.20 (*3*) between the greatest and least diameters of the fibers. It is not possible, however, to record the diameter at more than a limited number of points along the length of the fiber.

An extensive study of fiber-diameter measurements was conducted by Bosman (*4*) on South African Merino sheep. One thousand samples were selected at random, involving 250,000 fibers. The fibers were cut into small clippings at 8 to 10 places along the fiber length and mounted, and width measurements of the longitudinal clippings were made. Eighty percent of the samples were 60's to 100's, and 11 percent were 120's to 150's.

METHODS AND MATERIALS

The procedure used to secure the fiber-diameter measurements is a modification of the cross-section method recommended by the American Society for Testing Materials (*2*). Samples of wool fiber were taken from the shoulder, middle, and thigh at one side of each of five different breeds of sheep—Hampshire, Rambouillet, Shropshire, Southdown, and an experimental crossbred. These sheep were selected from the flock of the department of animal husbandry of South Dakota State College. Five sheep of the Hampshire, Southdown, and crossbred breeds were sampled in 1934, six of all breeds in 1935, and nine additional crossbreds in 1938. All samples were thoroughly cleaned with carbon tetrachloride and hand-carded to mix them thoroughly. Representative samples were taken from the cleaned fiber and stained with a saturated solution of picric acid. Hardy's device (*1*) was used in preparing the cross-sectional microscopic slides. All samples were prepared so that the fibers were measured at the midpoint of their fiber length. The cross-section mounts were placed on a microscopic stage set at right angles to the base, and by the use of strong artificial light their images were projected onto a ground-glass plate placed at such a distance from the microscope that each fiber section was magnified approximately 600 times. Two hundred fiber sections were measured with a calibrated wedge at their greatest and least diameters at right angles to each other and to an accuracy of 1 micron as recommended by the American Society for Testing Materials (*2*). From these results the average diameters and the relationships between the two dimensions were calculated.

In order to draw reliable conclusions, statistical methods were employed in the analyses of the data. The mean diameter and contour ratios were determined, and the standard deviation was calculated to furnish a measure of the variability. The analysis of variance as developed by Fisher (*7*) was used throughout this study. The χ^2 test for homogeneity of errors was applied by using the formula suggested by Snedecor (*15*).

EXPERIMENTAL RESULTS

In presenting the data of the five breeds of sheep studied, it is recognized that the results obtained are not necessarily representative of these breeds as they exist universally. It is felt, however, that the data are useful in indicating the type of variation in fiber diameter and fiber contour among individual sheep, within a breed group, which may be expected when working with material of a similar type.

The usefulness of the data for this discussion becomes apparent in studies of the type for which the material was originally intended; i. e., in the study of the wool fiber used in the manufacture of experimental fabrics from fleeces of known origin. It also serves to indicate differences which may be expected in fleeces of sheep of the same breed, and in fleeces from sheep of different breeds. Descriptions of breeds and the variations to be found may be of aid to the wool grader.

In table 1 are listed the sheep of each breed studied and the average diameter and average contour of 200 wool fibers from each of three locations on each sheep; i. e., shoulder, middle, and thigh. The variability of these measurements is reported as the standard deviations of single observations.

An examination of the data in table 1 shows that there were wide variations in fiber diameter and contour between sheep within a breed, even for a given location in the fleece. The correlation coefficients for testing the association between the means and standard deviations for fiber diameters within breeds at the various positions on the fleece were calculated and tested for homogeneity according to Rider's method (*11*) by means of the formula:

$$\chi^2 = S(n-3)Z^2 - \frac{(S(n-3)Z)^2}{S(n-3)}$$

where

$$Z = 1/2 \log_e \frac{1+r}{1-r}$$

These correlations were found to be homogeneous, and the average correlation obtained from the weighted mean of Z was $r = +0.371$ Since Z is 4.23 times its standard error it is highly significant, and the average r (0.371) must be highly significant also.

The standard deviation of the thigh portion of the fleece was lower than that in the other portions of the fleece studied in the case of one each of the Rambouillet and Shropshire fleeces. The Rambouillet fleeces were smaller, on the average, in diameter at the middle portion than at the shoulder; and the crossbred fleeces were very slightly smaller, on the average, in the shoulder than in the middle portion. The Shropshire fleeces throughout the three portions were most uniform of the breeds studied, and the Southdown fibers were appreciably larger at the thigh than at the shoulder and middle sections.

TABLE 1.—*Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep*

Breed and section of fleece	Sample No.	Average diameter	Standard deviation [1]	Average fiber-contour ratio	Standard deviation [1]
		Microns	*Microns*		
Hampshire: Shoulder	1-34	24.71	5.53	1.24	0.152
	1-35	26.72	5.94	1.31	.194
	2-35	26.80	6.06	1.23	.176
	2-34	27.74	7.09	1.30	.175
	3-35	29.09	6.13	1.36	.250
	4-35	29.23	4.59	1.36	.204
	3-34	31.02	4.89	1.27	.149
	4-34	31.88	6.16	1.28	.166
	5-35	34.06	7.04	1.37	.217
	6-35	34.96	6.24	1.36	.201
	5-34	36.89	5.71	1.42	.232
Average		30.28	5.991	1.32	.1951
Middle	1-34	26.26	5.97	1.23	.144
	1-35	28.16	6.61	1.34	.215
	2-35	29.97	5.38	1.28	.194
	2-34	29.61	7.60	1.30	.202
	3-35	36.05	6.43	1.46	.313
	4-35	32.94	5.71	1.40	.197
	3-34	38.68	9.06	1.33	.191
	4-34	33.49	6.14	1.31	.186
	5-35	33.64	6.30	1.38	.263
	6-35	33.38	6.11	1.42	.273
	5-34	35.47	6.10	1.36	.233
Average		32.51	6.56	1.35	.224
Thigh	1-34	29.20	6.54	1.27	.163
	1-35	29.52	7.15	1.32	.177
	2-35	31.28	6.52	1.34	.189
	2-34	37.78	9.78	1.40	.241
	3-35	38.05	7.97	1.47	.269
	4-35	36.59	7.98	1.44	.268
	3-34	38.46	8.00	1.36	.229
	4-34	35.86	7.27	1.33	.212
	5-35	37.84	8.99	1.43	.294
	6-35	41.10	6.39	1.38	.203
	5-34	38.28	10.42	1.31	.205
Average		35.81	8.32	1.37	.226
Rambouillet: Shoulder	1-35	19.82	2.86	1.26	.135
	2-35	21.26	3.88	1.22	.165
	3-35	22.68	4.51	1.25	.137
	4-35	23.84	5.29	1.27	.151
	5-35	24.42	3.76	1.28	.195
	6-35	26.46	3.73	1.33	.202
Average		23.08	4.07	1.27	.166
Middle	1-35	21.03	3.33	1.21	.118
	2-35	20.84	3.21	1.30	.163
	3-35	21.78	3.20	1.27	.152
	4-35	21.62	5.33	1.29	.193
	5-35	22.53	4.26	1.25	.151
	6-35	23.66	3.26	1.29	.166
Average		21.91	3.85	1.27	.158
Thigh	1-35	23.58	3.47	1.25	.135
	2-35	24.13	5.78	1.25	.164
	3-35	22.24	3.98	1.33	.223
	4-35	27.04	4.93	1.32	.228
	5-35	21.24	4.44	1.25	.174
	6-35	27.58	3.99	1.38	.240
Average		24.30	4.50	1.30	.198
Shropshire: Shoulder	1-35	28.24	7.10	1.43	.241
	2-35	29.19	6.80	1.30	.173
	3-35	30.26	7.27	1.36	.202
	4-35	30.00	6.93	1.34	.203
	5-35	32.99	7.38	1.38	.216
	6-35	35.50	6.78	1.49	.299
Average		31.03	7.05	1.38	.226

[1] Average standard deviations were obtained as an average within fleeces by averaging the squared standard deviations and extracting the square root.

TABLE 1.—*Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep*—Continued

Breed and section of fleece	Sample No.	Average diameter	Standard deviation	Average fiber-contour ratio	Standard deviation
		Microns	*Microns*		
Middle	1-35	32.52	6.24	1.47	0.234
	2-35	29.20	6.77	1.31	.233
	3-35	29.72	7.28	1.32	.184
	4-35	33.22	7.94	1.40	.266
	5-35	36.11	7.31	1.46	.282
	6-35	34.84	7.43	1.42	.243
Average		32.60	7.18	1.40	.242
Thigh	1-35	33.20	7.78	1.48	.262
	2-35	29.14	6.35	1.32	.205
	3-35	33.38	6.62	1.45	.275
	4-35	33.75	7.66	1.36	.211
	5-35	38.42	7.27	1.47	.263
	6-35	33.98	6.63	1.39	.238
Average		33.64	7.07	1.41	.244
Southdown: Shoulder	1-34	26.66	6.17	1.31	.199
	2-34	28.48	6.94	1.30	.202
	3-34	29.22	6.56	1.18	.107
	4-34	30.32	6.46	1.27	.213
	5-34	30.67	5.18	1.28	.174
	1-35	31.05	6.35	1.26	.183
	2-35	31.05	5.31	1.26	.187
	3-35	31.87	6.73	1.26	.162
	4-35	33.63	7.22	1.33	.209
	5-35	33.94	6.63	1.28	.174
	6-35	34.11	6.32	1.27	.172
Average		31.00	6.38	1.27	.182
Middle	1-34	30.00	7.55	1.28	.194
	2-34	30.43	7.28	1.27	.170
	3-34	30.81	6.78	1.22	.136
	4-34	30.62	6.26	1.26	.213
	5-34	31.30	6.36	1.26	.161
	1-35	32.24	6.84	1.31	.232
	2-35	31.52	5.60	1.32	.153
	3-35	31.44	7.44	1.29	.174
	4-35	30.72	6.68	1.28	.170
	5-35	32.19	6.72	1.29	.204
	6-35	35.25	7.18	1.25	.167
Average		31.50	6.81	1.27	.182
Thigh	1-34	33.30	8.16	1.32	.218
	2-34	33.00	8.01	1.36	.228
	3-34	33.08	8.98	1.26	.168
	4-34	30.78	7.39	1.28	.179
	5-34	33.42	8.90	1.29	.191
	1-35	40.66	10.18	1.34	.214
	2-35	35.34	8.56	1.27	.154
	3-35	39.04	11.31	1.29	.190
	4-35	42.39	9.42	1.40	.218
	5-35	36.12	12.27	1.35	.229
	6-35	49.95	12.66	1.36	.223
Average		37.01	9.77	1.32	.203
Experimental crossbred: Shoulder	1-34	21.13	3.40	1.19	.110
	2-34	24.34	4.35	1.20	.138
	1-35	24.43	4.40	1.26	.151
	2-35	24.54	4.85	1.29	.165
	1-38	24.85	4.93	1.23	.167
	2-38	24.86	4.23	1.27	.149
	3-38	26.10	4.51	1.24	.153
	3-34	26.55	6.04	1.27	.165
	3-35	26.84	5.54	1.32	.221
	4-35	26.93	4.04	1.27	.170
	5-35	26.94	4.62	1.27	.165
	4-34	27.01	5.80	1.25	.170
	4-38	27.28	5.88	1.24	.146
	5-38	27.84	3.56	1.22	.139
	6-35	28.83	5.54	1.33	.206
	6-38	29.33	4.81	1.28	.143
	5-34	29.72	6.18	1.26	.159

TABLE 1.—*Average diameter, average contour ratios, and standard deviations of single measurements of 200 wool fibers from each of 3 sections of fleeces from 5 breeds of sheep*—Continued

Breed and section of fleece	Sample No.	Average diameter	Standard deviation	Average fiber-contour ratio	Standard deviation
		Microns	*Microns*		
Experimental crossbred—Continued	7-38	30.36	4.94	1.21	0.112
Shoulder	8-38	30.52	5.28	1.23	.136
	9-38	31.82	5.66	1.29	.195
Average		27.01	4.99	1.25	.160
	1-34	23.96	4.64	1.27	.190
	2-34	26.24	4.46	1.22	.152
	1-35	26.90	4.99	1.28	.156
	2-35	24.34	4.78	1.31	.178
	1-38	24.14	4.62	1.23	.140
	2-38	26.23	5.01	1.31	.169
	3-38	27.37	5.39	1.28	.172
	3-34	27.08	7.51	1.29	.200
	3-35	29.60	7.04	1.32	.202
Middle	4-35	27.40	4.84	1.26	.166
	5-35	27.15	4.47	1.31	.189
	4-34	26.82	7.13	1.25	.146
	4-38	29.12	4.11	1.28	.183
	5-38	26.83	5.55	1.27	.142
	6-35	24.75	5.92	1.30	.214
	6-38	28.72	5.05	1.27	.149
	5-34	27.65	5.30	1.27	.182
	7-38	28.96	5.84	1.25	.148
	8-38	28.22	4.50	1.21	.117
	9-38	31.77	5.70	1.29	.162
Average		27.13	5.42	1.27	.170
	1-34	26.04	6.43	1.31	.219
	2-34	31.11	8.59	1.28	.193
	1-35	31.40	6.95	1.34	.230
	2-35	31.28	9.74	1.38	.258
	1-38	27.32	6.88	1.24	.141
	2-38	27.40	8.10	1.31	.197
	3-38	28.06	6.90	1.28	.154
	3-34	32.61	13.06	1.33	.253
	3-35	39.92	14.46	1.45	.281
Thigh	4-35	32.65	7.91	1.44	.270
	5-35	35.88	8.62	1.43	.265
	4-34	32.47	11.48	1.27	.195
	4-38	25.98	7.87	1.28	.189
	5-38	35.00	6.82	1.33	.203
	6-35	31.98	7.59	1.37	.255
	6-38	30.44	6.44	1.35	.192
	5-34	33.74	10.67	1.33	.233
	7-38	31.58	11.17	1.29	.163
	8-38	34.38	7.87	1.31	.204
	9-38	31.37	5.99	1.31	.190
Average		31.53	8.98	1.33	.218

When the ratios between the two diameters of the fiber were calculated, it was found that in most instances the values for contour were appreciably higher than that given by Barker (*2*) for the ideal fiber. This was particularly marked in the case of the Shropshire fleeces. With the exception of the crossbred, approximately half of the fleeces showed progressively increasing contour ratios as the average fiber diameter of the three portions of the fleeces increased, while in the remaining half there was no relationship between these two factors. In the case of the crossbred fleeces, two-thirds of the fleeces exhibited increasing rations as the diameters increased. In any one breed, however, the fleeces with the lowest diameters did not necessarily have the lowest fiber-contour ratios.

In order to determine whether the variability of the fiber diameter or contour measurements for separate locations on the fleeces of in-

dividual sheep within a breed were homogeneous, the χ^2 test for homogeneity of errors, given by Snedecor (*15*), was applied to the data. The results are given in table 2.

TABLE 2.—*Analyses of errors of diameter and countour measurements for the different breeds of sheep by means of the χ^2 test for homogeneity*

Fiber measurements and breed	Degrees of freedom	χ^2 for—		
		Shoulder	Middle	Thigh
Diameter:				
Hampshire	10	[1] 65.91	[1] 118.62	[1] 120.92
Rambouillet	5	[1] 83.16	[1] 122.75	[1] 68.77
Shropshire	5	2.53	[2] 12.91	[2] 14.44
Southdown	10	[1] 34.86	[1] 26.82	[1] 140.63
Experimental crossbred	19	[1] 186.93	[1] 269.74	[1] 639.99
Contour ratios:				
Hampshire	10	[1] 109.57	[1] 196.63	[1] 138.42
Rambouillet	5	[1] 61.54	[1] 44.66	[1] 83.71
Shropshire	5	[1] 84.53	[1] 35.26	[1] 27.48
Southdown	10	[1] 81.54	[1] 124.54	[1] 61.01
Experimental crossbred	19	[1] 249.61	[1] 155.75	[1] 249.61

[1] Exceeds the 1-percent level of significance.
[2] Exceeds the 5-percent level of significance.

It is seen that χ^2, when applied to the error variances of the diameter measurements, is significant in all instances except at the shoulder in the Shropshire breed. The fleeces representing the Shropshire breed were the most homogeneous, and those of the crossbred the most heterogeneous. In the Southdown breed considerably greater variation was found in the thigh than in the shoulder and middle portions of the fleeces.

When the χ^2 test was applied to the error variances of the contour measurements of these same fleeces represented by the same fibers as were measured for diameter, the results again indicated that all of the fleeces representing each breed were heterogeneous. If all of the breeds are considered, it is noted that no one portion of the fleeces was more homogeneous than the others throughout the series. In two of the breeds the thigh sections were most homogeneous; in two others, the middle; and in the fifth breed, the shoulder sections of the fleeces.

It is apparent that variability between both fiber diameter or contour measurements of different sheep within a breed was greater than could be explained by chance, when 200 fibers were measured at a given location, except for diameter at the shoulder portion of the Shropshire breed, where only six sheep were involved in the test.

In the calculations of analyses of variance, homogeneity of the errors of the source material is assumed. As is shown in table 2, the fleeces of the five breeds studied did not exhibit this characteristic. Since, however, the data on homogeneity are the only ones which were available to describe the breed, they have been employed for the analyses of the diameter and contour measurements of the sheep within breeds. It was recognized, however, that the variability of the individual fiber measurements of different sheep was not of a homogeneous nature. In table 3 are shown the mean squares for sheep and fibers within fleeces (error) of the diameter and contour measurements. Differences between fiber measurements of the sheep within a breed were highly significant in all instances.

Table 3 shows that in the case of the mean square values for variation due to error, the values for the diameter measurements increased in progressing from the shoulder to the middle to the thigh in the Hampshire, Southdown, and crossbred breeds. The errors were lower in the middle portions of the Rambouillet, and practically the same in the three sections of the Shropshire breed. Because of the differences in mean square values for the various portions of the several breeds, it is not possible to group them and report one value as characteristic of the breed. The only instance where it would be possible to report one value for breed is in the case of the Shropshire.

The same type of relationships between the errors of the shoulder, middle, and thigh sections of the various breeds is found in the fiber-contour ratios, also shown in table 3.

TABLE 3.—*Analyses of variance of the diameters and contour ratios of wool fibers from 5 breeds of sheep, variability between sheep being measured within years*

Section of fleece and breed	Fiber diameter				Fiber contour ratios			
	Variation due to sheep		Variation due to error		Variation due to sheep		Variation due to error	
	Degrees of freedom	Mean square [1]	Degrees of freedom	Mean square	Degrees of freedom	Mean square [1]	Degrees of freedom	Mean square
Shoulder:								
Hampshire	9	3,279.89	2,189	35.85	9	0.75784	2,189	0.03801
Rambouillet	5	1,112.00	1,194	16.59	5	.27286	1,194	.02770
Shropshire	5	1,437.00	1,194	49.68	5	.90560	1,194	.05105
Southdown	9	461.67	2,189	40.69	9	.56926	2,189	.03327
Experimental crossbred	17	1,265.06	3,979	24.89	17	.18208	3,979	.02362
Middle:								
Hampshire	9	3,000.78	2,189	43.09	9	.64577	2,189	.05008
Rambouillet	5	217.40	1,194	14.80	5	.22482	1,194	.02514
Shropshire	5	1,503.80	1,194	51.58	5	1.14100	1,194	.05874
Southdown	9	298.56	2,189	46.43	9	.15821	2,189	.03300
Experimental crossbred	17	708.29	3,979	29.39	17	.15758	3,979	.02872
Thigh:								
Hampshire	9	3,520.44	2,189	64.19	9	.64871	2,189	.05122
Rambouillet	5	1,296.20	1,194	20.21	5	.62344	1,194	.03909
Shropshire	5	1,739.20	1,194	50.04	5	.77926	1,194	.05946
Southdown	9	3,237.00	2,189	95.45	9	.38293	2,189	.04108
Experimental crossbred	17	2,087.88	3,979	81.42	17	.32276	3,979	.04710

[1] All F values exceed 1-percent point.

The ratio of variation due to sheep to variation due to error (F) was proportionally higher for diameters than for contour. Thus, while in the contour studies, all of the values for F were highly significant at the 1-percent point, the results were lower than those found in the case of the diameter. The sheep varied relatively less in contour of fiber than in diameter. As was shown for the diameter studies, it is impossible to assign one value for contour as characteristic of the breed except in the case of the Shropshire.

In table 4 are given comparisons of the differences between mean fiber diameters and mean contour ratios of the different breeds for a specified location on the fleece. For such comparisons the mean squares for sheep within breeds were used to determine the significance of differences between the means of different breeds. The t test (7) was used for testing the significance of such differences.

TABLE 4—*Mean differences* [1] *and their standard errors for fiber diameter and fiber contour of wool from 5 breeds of sheep clipped in 1935*

Section of fleece and breed	Data for fiber diameter of breed indicated				Data for fiber contour of breed indicated			
	Rambouillet	Shropshire	Southdown	Crossbred	Rambouillet	Shropshire	Southdown	Crossbred
Shoulder:								
Hampshire	[2] +7.06±1.74	−1.22±1.82	−2.47±1.57	+3.72±1.61	+0.03±0.026	−0.05±0.035	+0.05±0.024	+0.04±0.025
Rambouillet		[2] −8.28±1.46	[2] −9.53±1.13	[2] −3.34±1.18		−.11± .031	−.01± .018	−.02± .019
Shropshire			−.25±1.24	[2] +4.94±1.29			[2] +.10± .029	[2] +.09± .030
Southdown				[2] +6.19± .90				−.01± .016
Middle:								
Hampshire	[2] +10.45±1.23	−.24±1.61	+.14±1.32	[2] +5.77±1.36	+.11± .029	−.02± .040	[2] +.09± .029	[2] +.08± .028
Rambouillet		[2] −8.28±1.17	−10.31±7.73	[2] −4.68± .83		[2] −.13± .034	[2] −.02± .019	−.03± .017
Shropshire			+.38±1.29	[2] +6.01±1.33			[2] +.11± .016	[2] +.10± .014
Southdown				[2] +5.63± .96				−.01± .016
Thigh:								
Hampshire	[2] +11.43±2.08	+2.09±2.17	−4.85±2.82	+1.88±2.28	[2] +.10± .034	−.01± .036	+.07± .035	[2] +.00± .028
Rambouillet		[2] −9.34±1.58	[2] −16.28±2.40	[2] −9.55±1.74		[2] −.11± .029	−.03± .033	[2] −.10± .029
Shropshire			[2] −6.94±2.48	−.21±1.84			[2] +.08± .027	+.01± .031
Southdown				[2] +6.73±2.58				−.07± .030

[1] The differences were obtained by subtracting the means of the breeds listed in the box heads from the means of the breeds in the column.
[2] Exceeds 1 percent. in the *t* test.
[3] Exceeds 5 percent. in the *t* test.

In the case of the differences between means of the diameter measurements, the differences were found to exceed the 1-percent point in the *t* test where comparisons between the Hampshire and Rambouillet and the Shropshire and Rambouillet fleeces at the shoulder, middle, and thigh; the Southdown and Rambouillet at the shoulder and thigh; the Southdown and crossbred at the shoulder and middle; the crossbred and Rambouillet at the thigh and middle; and between the crossbred and all other breeds at the middle portions of the fleeces. The Rambouillet and crossbred, and the Shropshire and crossbred were significantly different at the 5-percent point at the shoulder, and the Shropshire and crossbred and the Shropshire and Southdown at the thigh portion.

When the contour ratios were studied, the differences were significant at the 1 percent point in comparisons of the Rambouillet and Shropshire, the Shropshire and Southdown, the Shropshire and crossbred, and the Rambouillet and Southdown at the middle portion of the fleeces; and the Rambouillet and Shropshire, and the Hampshire and crossbred at the thigh. The Shropshire and Southdown and the Shropshire and crossbred showed significant differences between means at the 5-percent point at the shoulder portions; the Hampshire and Southdown, and the Hampshire and crossbred at the middle; and the Shropshire and Southdown, the Rambouillet and crossbred, and the Hampshire and Rambouillet at the thigh portion of the fleeces.

The accuracy with which the mean fiber diameter and contour of a breed can be specified depends on two components, the variability among sheep within a breed and the number of fibers measured per sheep. To demonstrate this relationship the data of the fibers from the shoulder section of the crossbred sheep are employed. The crossbred was chosen because a greater number of measurements were made for this breed and the values of the mean squares for error were relatively low. The mean error variance of the breed was 1,265.06 (table 3) divided by 4,000 or 0.3163. From these data the number of fibers necessary to measure from each fleece, if the number of fleeces were doubled, was then estimated (table 5). The formula used was one given by Immer (*9*).

TABLE 5.—*Estimate of number of fibers to be measured on shoulder section of crossbred breed when mean error of breed is specified*

Measurement	Mean error for breed	Sheep to be measured	Fibers required from each sheep
		Number	*Number*
Average fiber diameter	0.3163	20	200.0
	.3163	40	3.9
Average fiber contour ratio	.0000455	20	200.0
	.0000455	40	26.0

The great variability between the means of the sheep compared with the comparatively much lower variability between fibers on the same sheep permits a much reduced number of fibers per sheep as the number of sheep is increased, in describing the breed fiber characteristics.

SUMMARY

The diameters of samples of wool fiber from fleeces representing five breeds of sheep were measured in cross section and their contour ratios were calculated. Wide variations in diameter between sheep within a breed, and, in most instances, between breeds were found.

Contour ratios were found, in general, to be higher than the ratio of 1:1.20 given by Barker for the ideal fiber. With the exception of the crossbred, in approximately half of the cases fiber contour was found to increase positively as fiber diameter of the various portions of a single fleece increased. Within a breed the lowest contour ratios were not necessarily found for those fleeces having the smallest diameters. In three of the breeds a majority of the fleeces showed increasing standard deviation as the contour increased. In the other two breeds, no relationships were found.

χ^2 tests for homogeneity of fleeces within a breed showed that when 200 fibers were measured per fleece it could be demonstrated that in all instances heterogeneous groups of sheep composed the samples used to represent the breeds.

In the analyses of variance for the diameter and contour measurements of sheep within breeds, F was found to be highly significant at the 1-percent point in all instances but one. Because of the variation in mean square values for error, it was not possible to report a single value for the breed, with the possible exception of the Shropshire breed.

Calculations of the number of fibers necessary to measure to secure the same error for the breed were made, using the shoulder measurements of the crossbred as an example. It was found that the fibers necessary would decrease rapidly if the number of sheep were increased.

LITERATURE CITED

(1) ANONYMOUS.
1935. NEW CROSS-SECTIONING DEVICE. Textile Res. 5: 580.

(2) AMERICAN SOCIETY FOR TESTING MATERIALS.
1938. A. S. T. M. STANDARDS ON TEXTILE MATERIALS. Prepared by Committee D–13 on Textile Materials. 323 pp., illus. Philadelphia.

(3) BARKER, S. G.
1931. WOOL QUALITY: A STUDY OF THE INFLUENCE OF VARIOUS CONTRIBUTORY FACTORS, THEIR SIGNIFICANCE AND THE TECHNIQUE OF THEIR MEASUREMENTS. 333 pp., illus. London

(4) BOSMAN, V.
1934. THE FIBRE FINENESS OF SOUTH AFRICAN MERINO WOOL. Onderstepoort Jour. Vet. Sci. and Anim. Indus. 3: 223–231, illus.

(5) BURNS, ROBERT H.
1935. THE MICROMETER CALIPER FOR MEASURING THE THICKNESS OF WOOL FIBERS. Wyo. Agr. Expt. Sta. Bul. 204, 36 pp., illus.

(6) ——— and KOEHLER, W. B.
1925. THE MICROMETER CALIPER AS AN INSTRUMENT FOR MEASURING THE DIAMETER OF WOOL FIBERS. Wyo. Agr. Expt. Sta. Bul. 141, pp. [15]–28, illus.

(7) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

(8) HARDY, J. I.
1935. DETERMINATION OF FIBRE FINENESS AND CROSS-SECTIONAL VARIABILITY. AN IMPROVED TECHNIQUE FOR OBTAINING PHOTOMICROGRAPHS BY PROJECTING CROSS-SECTIONS ON SENSITIZED PAPER. Textile Res. 5: 184–190, illus.

235081—40——5

(9) IMMER, F. R.
1932. A STUDY OF SAMPLING TECHNIC WITH SUGAR BEETS. Jour. Agr. Res. 44: 633–647.

(10) KRAUTER, G.
1929. ÜBER DIE WOLLFEINHEITMESSUNG. Leipzig. Monatschr. f. Textil-Indus. 44: 1–6, 45–52, 89–96, illus. Also in Textile Forschung 1: 1–67. [Original not seen. Abstract in Textile Inst. Jour. 22: A217. 1931.]

(11) RIDER, PAUL R.
1939. AN INTRODUCTION TO MODERN STATISTICAL METHODS. 220 pp., illus. New York and London.

(12) ROBERTS, J. A. FRASER.
1930. FLEECE ANALYSIS FOR BIOLOGICAL AND AGRICULTURAL PURPOSES. THE AVERAGE FINENESS OF A SAMPLE OF WOOL. Textile Inst. Jour. 21: T127–T165, illus.

(13) SCHWARZ, E. R.
1936. IMPROVED RAPID SECTIONING OF FIBRES. Textile Res. 6: 270–272, illus.

(14) SIDEY, D. J.
1931. SOME RESULTS OF CROSS-SECTIONAL AREA AND CONTOUR MEASUREMENTS OF NEW ZEALAND ROMNEY AND CORRIEDALE WOOLS. Textile Inst. Jour. 22: T299–T304, illus.

(15) SNEDECOR, GEORGE W.
1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. 341 pp., illus. Ames, Iowa.

(16) STANBURY, G. R., and DANIELS, H. E.
1937. A COMPARATIVE EXAMINATION OF METHODS OF ANALYSIS OF WOOL FOR FIBRE DIAMETER AND WEIGHT. PART I. DIAMETER MEASUREMENTS IN WOOL TOPS. Textile Inst. Jour. 28: T188–T201.

(17) WILDMAN A. BLAKEY.
1936. SOME CHARACTERISTICS WHICH ENTER INTO THE ASSESSMENT OF WOOL QUALITY, AND THEIR ESTIMATION IN THE FLEECE. Textile Inst. Jour. 27: P181–P196, illus.

(18) ——— and DANIELS, H. E.
1937. A COMPARATIVE EXAMINATION OF METHODS OF ANALYSIS OF WOOL FOR FIBRE DIAMETER AND WEIGHT. PART II. THE ANALYSIS OF RAW WOOL FOR FIBRE FINENESS. Textile Inst. Jour. 28: T202–T204.

EFFECTS OF LOW TOPPING AND DIASTATIC MALT EXTRACT ON COMPOSITION AND QUALITY OF SORGO SIRUP [1]

By C. F. WALTON, JR., *senior chemist*, E. K. VENTRE, *chemist*, and S. BYALL, *associate chemist, Carbohydrate Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture* [2]

INTRODUCTION

The present lack of uniformity in the quality of sorgo sirup may be attributed to (1) the characteristic differences in composition of juice from different varieties and from different parts of the stalk, (2) the effect of soils and fertilizers, (3) the influence of maturity and seasonal conditions, and (4) the kind of equipment, procedure, and skill employed in making the sirup. Some of these factors have previously been studied, special attention having been given to the variable composition of different parts of the sorgo stalk at several stages of maturity [3] and the relationships between the content of starch, sucrose, and reducing sugar and the jellying and crystallizing characteristics of the sirups.[4] Since the authors have reviewed the work of other investigators in earlier publications on this subject, there is no need for a detailed discussion of the literature here.

Briefly, it has been found that sirups made from the upper part of the sorgo stalk usually contain a greater percentage of starch, sucrose, mineral matter, and acidity, in proportion to dissolved solids, than do those made from the rest of the stalk. Results reported previously also show that the composition of the stalk varies considerably at different stages of maturity, the dough-to-ripe stage being considered best for sirup making. It has been found that starch is principally responsible for difficult filtration of the juice and for slow boiling, scorching, and jellying of the sirup. It has also been found that both sucrose and dextrose crystallization occur in sirups produced from different parts of the stalk of several varieties of sorgo at different stages of maturity.

DEVELOPMENT OF MALT-EXTRACT METHOD

Investigations of malt extracts and other commercial preparations containing starch-hydrolyzing enzymes were made in 1932 for the purpose of ascertaining what types of products of high starch-converting activity were available commercially at low prices. In the fall of that year, a practicable farm-scale method was developed for using malt extract of high diastatic activity on cold sorgo juice freshly expressed from the mill.[5]

---

[1] Received for publication Mar. 6, 1940.

[2] The authors gratefully acknowledge the cooperation of Martin Nelson and C. K. McClelland, of the Agronomy Department, University of Arkansas, and also that of the late J. R. Ricks, director, and the late W. R. Perkins, assistant director, of the Mississippi Agricultural Experiment Station, in supplying working facilities and in connection with the agronomic work of this investigation.

[3] VENTRE, EMIL K., and BYALL, S. DISTRIBUTION AND VARIATION WITH MATURITY OF DISSOLVED SOLIDS, SUCROSE, AND TITRATABLE ACIDITY IN THE SORGO STALK. Jour. Agr. Res. 55: 553–562. 1937.

[4] ——BYALL, S., and WALTON, C. F., JR. JELLYING AND CRYSTALLIZATION OF SIRUPS MADE FROM DIFFERENT PARTS OF THE SORGO STALK AT DIFFERENT STAGES OF MATURITY. Jour. Agr. Res. 59:139–150. 1939.

[5] UNITED STATES BUREAU OF CHEMISTRY AND SOILS. SORGHUM SIRUP. HOW TO PREVENT JELLYING AND SLOW BOILING, AND HOW TO PREVENT SUGARING. U. S. Bur. Chem. and Soils Cir., 4 pp. 1933. [Mimeographed.]

During the season of 1934, in cooperation with the Arkansas Agricultural Experiment Station, the use of high-diastatic malt extract on sorgo juice was compared with its use on the semisirup. A severe drought during the growing season of 1934 adversely affected both the yield and the quality of all sorgo on the experimental plots The juices from all varieties contained such a high percentage of starch that the sirups invariably scorched in the evaporator before final density was reached, except when malt-extract treatment was applied. Incidentally, on standing overnight many of the untreated sirups also clabbered or jellied. Scorching, however, rather than clabbering, was taken as the index of the efficacy of the starch-hydrolyzing treatment in the experimental work to develop the best procedure, since scorching could be noted at once while the sirups were being made. The proportion of malt extract used and the density and temperature of the semisirup to which it was added were varied until it was found under what conditions scorching could always be avoided. Similar tests were conducted on the use of malt extract in the juice. Control tests, in which no malt extract was used, were made on aliquots of the juice from all varieties. The exceptionally favorable conditions under which these experiments were carried on, particularly the uniformly high starch content of the juices, led to the development of a satisfactory method of using high-diastatic malt extract that can be depended on to yield good results, even with sorgo of extremely high starch content. The methods of using malt extract on the juice and on the semisirup have been described recently.[6] The semisirup method is preferred to the juice method.

The objects of the present investigation were to compare the composition and quality of sirups made from the whole and from the topped stalks by a standard procedure with those of sirups made by the process in which high-diastatic malt extract was used, and to evaluate the benefits of the low-topping procedure in comparison and in conjunction with those obtained by the use of high-diastatic malt extract.

MATERIALS AND METHODS

The research was conducted in cooperation with the Mississippi Agricultural Experiment Station, eight varieties of sorgo grown by the station being utilized for this work. In general, each variety was harvested at two or three stages of maturity, and sirup was made from the whole stalks and from the topped stalks by a standard method, as a control, for comparison with sirups made by modifications of the standard procedure. In collecting the topped-stalk samples of sorgo, from two to four of the upper joints below the peduncle, depending on the length of the stalk, were discarded.

The equipment and procedure designated as standard have been described in a previous publication.[7] In producing sirups by the standard method the juice was allowed to settle for 2 hours and was then decanted and boiled, with continuous skimming, to the density of finished sirup. The second series of samples was made by the same procedure, except that evaporation was discontinued when the density

[6] WALTON, C. F., JR., VENTRE, E. K., and BYALL, S. FARM PRODUCTION OF SORGO SIRUP. U. S. Dept. Agr. Farmers' Bul. 1791, 40 pp., illus. 1938.

[7] WALTON, C. F. JR., and VENTRE, E. K. EVAPORATOR FOR RESEARCH ON SIRUP MANUFACTURE. Internatl. Sugar Jour. 39: 430–431. 1937.

reached 20° Baumé, measured at close to the boiling temperature, and the semisirup was allowed to settle overnight, after which it was returned to the evaporator and evaporated to final density, with the usual careful skimming. In making the third series of samples, the high-diastatic malt-extract method previously developed for treating the semisirup was used.

Duplicate samples of the finished sirup were filled when hot, direct from the evaporator, into 4-ounce screw-top bottles. Soon after the end of the sirup-making season they were analyzed for density, sucrose, invert sugar, ash, and starch. After standing for over a year, they were examined also for flavor, color, turbidity, clabbering or jellying, and crystallization.

RESULTS

The analytical data and observations regarding the quality of the sirups are given in table 1. The percentages of the components mentioned are all expressed as percentages of the weight of solids determined at sirup density by refractometer.

TABLE 1.—*Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity*

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids [1]				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
		Percent	*Percent*	*Percent*	*Percent*	
SILVER TOP (DOUGH-TO-RIPE)						
Tops	Standard	61.43	28.52	2.28	1.57	Jellied.
Whole stalk	do	52.70	42.05	2.26	.67	Very cloudy.
	Semisirup settled	46.80	45.97	2.30	.90	Do.
	Malt extract used on semisirup.	49.72	45.09	2.23	.38	No jellying and settled clear.
Topped stalk	Standard	57.15	38.15	2.10	1.02	Very cloudy.
	Semisirup settled	57.32	39.88	1.96	.77	Do.
	Malt extract used on semisirup.	57.28	38.12	2.14	.22	Settled clear, with no jellying.
SILVER TOP (OVERRIPE)						
Whole stalk	Standard	67.46	31.75	1.90	1.00	Very cloudy.
	Semisirup settled	60.08	35.01	1.96	1.38	Do.
	Malt extract used on semisirup.	58.01	37.59	1.83	.34	Settled clear, with no jellying.
Topped stalk	Standard	54.07	40.02	1.76	1.11	Very cloudy.
	Semisirup settled	55.15	38.61	1.85	1.24	Do.
	Malt extract used on semisirup.	54.11	41.73	1.68	.23	Settled fairly clear, with no jellying.
RED X (DOUGH-TO-RIPE)						
Tops	Standard	66.35	26.94	4.63	2.08	Jellied.
Whole stalk	do	68.28	23.16	3.15	1.92	Do.
	Semisirup settled	69.26	22.44	3.09	1.83	Do.
	Malt extract used on semisirup.	66.32	25.25	2.88	.40	No jellying, and settled fairly clear.
Topped stalk	Standard	68.54	22.80	2.83	2.03	Jellied.
	Semisirup settled	70.00	23.93	2.84	1.49	Do.
	Malt extract used on semisirup.	66.54	26.11	2.67	.82	No jellying, and settled fairly clear.

[1] Solids determined by refractometer.

TABLE 1.—*Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity*—Continued

LEOTI RED (DOUGH-TO-RIPE)

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
		Percent	*Percent*	*Percent*	*Percent*	
Tops	Standard	64.75	22.30	3.91	2.84	Jellied.
Whole stalk	do	49.12	40.78	2.56	2.15	Do.
	Semisirup settled	44.85	49.57	2.57	1.30	Do.
	Malt extract used on semisirup.	47.20	44.00	2.31	.29	No jellying.
Topped stalk	Standard	42.23	48.26	2.04	1.40	Jellied.
	Malt extract used on semisirup.	47.22	44.82	1.97	.38	No jellying.

ICEBERG (RIPE)

Whole stalk	Standard	63.06	30.05	1.93	1.25	Much fine grain (sucrose crystallization due to extra high density).
	Semisirup settled	64.63	30.14	1.75	1.08	Cloudy.
	Malt extract used on semisirup.	64.20	32.19	1.66	.38	No jellying; no sugaring; settled clear.
Topped stalk	Standard	53.73	42.51	1.75	.65	Cloudy.
	Semisirup settled	53.00	42.15	1.68	.88	Do.
	Malt extract used on semisirup.	51.68	44.85	1.76	.30	No jellying; no sugaring; settled clear.

ICEBERG (OVERRIPE)

Whole stalk	Standard	72.30	21.46	2.22	1.46	Jellied solid, with 2 large sucrose crystals on top.
	Malt extract used on semisirup.	69.22	26.01	2.18	.33	No jellying; settled clear; rock-candy crystallization.
Topped stalk	Standard	62.09	30.25	2.23	1.66	Jellied solid.
	Malt extract used on semisirup.	62.80	33.73	2.15	.26	No jellying; settled clear.

KANSAS ORANGE (DOUGH-TO-RIPE)

Tops	Standard	53.28	34.16	4.28	1.28	Jellied.
Whole stalk	do	52.97	39.97	2.96		
	Semisirup settled	43.47	51.08	2.39	1.02	Started to jelly.
	Malt extract used on semisirup.	42.97	51.44	2.29	.36	No jellying; no sugaring; very clear.
Topped stalk	Standard	67.24	24.68	2.17	1.49	Jellied, with sucrose crystallization.
	Malt extract used on semisirup.	65.23	28.64	2.19	.58	No jellying; very clear; sucrose crystallization.

JAPANESE SEEDED RIBBON (RIPE)

Whole stalk	Standard	28.46	66.69	2.09	0.56	Cloudy.
	Semisirup settled	25.66	68.57	2.08	.53	Do.
	Malt extract used on semisirup.	23.98	69.60	2.08	.23	Clear.
Topped stalk	Standard	15.31	79.22	1.86	.30	Cloudy.
	Semisirup settled	18.65	77.98	1.98	.35	Do.
	Malt extract used on semisirup.	17.84	78.42	1.91	.24	Clear and dextrose crystallization.

TABLE 1.—*Effect of topping and method of manufacture on the quality of sirups made from different varieties of sorgo at different stages of maturity*—Continued

JAPANESE SEEDED RIBBON (DEAD RIPE)

Part of stalk used for sirup	Method of making sirup	Composition of the sirup solids				Observations
		Sucrose	Reducing sugars expressed as invert sugar	Ash	Starch	
		Percent	*Percent*	*Percent*	*Percent*	
Tops	Standard	43.69	50.00	3.50	1.02	Opaque and greenish.
Whole stalk	do	39.81	55.49	1.86	1.02	Cloudy.
	Malt extract used on semisirup.	40.76	53.62	1.84	.32	Settled clear.
Topped stalk	Standard	24.89	64.05	1.55	.72	Cloudy.
	Semisirup settled	25.16	71.51	1.64	.45	Do.
	Malt extract used on semisirup.	20.31	75.34	1.59	.27	Clear.

GOOSENECK (RIPE)

Whole stalk	Standard	51.03	41.32	2.25	1.01	Opaque and very viscous.
	Semisirup settled	51.76	40.73	2.16	1.03	Do.
	Malt extract used on semisirup.	51.96	42.34	1.95	.34	Settled clear and low viscosity.
Topped stalk	Standard	40.99	52.01	2.04	.62	Opaque and viscous.
	Malt extract used on semisirup.	42.69	52.58	1.73	.13	Settled clear and low viscosity.

GOOSENECK (OVERRIPE)

Whole stalk	Standard	57.95	34.01	2.46	1.22	Jellied.
	Malt extract used on semisirup.	59.17	35.68	2.22	.37	Settled clear and low viscosity.
Topped stalk	Standard	49.48	43.88	2.30	.97	Opaque and viscous.
	Malt extract used on semisirup.	47.57	46.03	2.04	.14	Settled clear and low viscosity.

HODO (GREEN)

Whole stalk	Standard	26.25	69.18	2.53	(1)	Slightly cloudy.
	Semisirup settled	27.18	67.35	2.49	(1)	Do.
	Malt extract used on semisirup	25.84	69.03	2.49	(1)	Clear.
Topped stalk	Standard	30.35	64.53	2.46	(1)	Slightly cloudy.
	Semisirup settled	30.48	64.79	2.46	(1)	Do.
	Malt extract used on semisirup	30.02	64.40	2.40	(1)	Clear.

HODO (RIPE)

Whole stalk	Standard	48.23	46.42	2.43	0.25	Cloudy.
	Semisirup settled	46.60	47.98	2.24	.16	Slightly cloudy.
	Malt extract used on semisirup	46.60	47.98	2.19	(1)	Clear.
Topped stalk	Standard	48.23	45.82	2.38	.15	Slightly cloudy.
	Semisirup settled	47.13	46.66	2.36	.11	Do.
	Malt extract used on semisirup	47.30	46.94	2.36	(1)	Clear.

HODO (OVERRIPE)

Tops	Standard	40.20	53.21	3.47	0.34	Very cloudy.
Whole stalk	do	52.78	41.84	2.65	.20	Cloudy.
	Semisirup settled	49.11	45.06	2.60	.12	Do.
	Malt extract used on semisirup	49.31	44.98	2.56	(1)	Settled clear.
Topped stalk	Standard	46.13	47.94	2.26	.08	Cloudy.
	Semisirup settled	42.23	52.17	2.25	.08	Slightly cloudy.
	Malt extract used on semisirup	42.35	52.33	2.19	(1)	Clear.

[1] Negative.

DISCUSSION

Some of the samples exhibited sucrose crystallization when the sucrose content was approximately 65 percent, or higher, and the density was higher than 78° Brix, while one with high invert sugar content showed dextrose crystallization. As the sirup samples varied somewhat in density, however, little attention was given to crystallization, either as a varietal characteristic or as related to the composition of the sirups.

Most of the sirups with a starch content of 1.25 percent or higher jellied or became extremely viscous, whereas those made by the use of high-diastatic malt extract had a starch content considerably under 1.0 percent, in many cases only about 0.25 percent, and did not jelly.

The ash content of the samples varied considerably with the variety but it was usually higher in sirups made from the top portion of the stalk. As a rule, sirup from the tops likewise contained a greater proportion of starch.

In making sirups with juice from the tops alone, it was observed that scorching usually occurred before evaporation to the standard density of sirup was completed. This is characteristic of juices of high starch content, and accounts for the dark color and strong flavor of some sirups.

Whenever diastatic malt extract was used in proper amount, the resulting sirup possessed greatly improved clarity, in comparison with the corresponding untreated sample, and also deposited its sediment more rapidly. The relatively high starch content of some sirups doubtless acts as a stabilizer, holding other nonsugars in suspension and giving a more turbid product.

SUMMARY AND CONCLUSIONS

Sirups made by a standard procedure from the whole stalks and from the topped stalks of eight varieties of sorgo were compared with sirups made by modifications of this procedure, special attention being given to the use of starch-hydrolyzing enzymes.

The topped stalks consistently produced sirup of better quality than the whole stalks, which, in turn, gave better sirup than did the tops alone.

Relatively little improvement in quality resulted from simply allowing the semisirup to settle, without malt-extract treatment, before completing the evaporation.

All sirups produced by the process in which high-diastatic malt extract was used were of better color, flavor, and clarity than the sirups made from corresponding parts of the stalk by the standard procedure.

The results show that sirups of the highest quality are produced by using starch-hydrolyzing enzymes to supplement reasonably good topping practice.

U. S. GOVERNMENT PRINTING OFFICE: 1940